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# Perception of emotions in small ruminants



THE UNIVERSITY  
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# Declaration

I declare that I have composed this thesis and that the research described in it is my own work, unless otherwise acknowledged. The work described has not been submitted for any other degree or professional qualification.

Lucille Gabrielle Anna Bellegarde

August 2017

# Abstract

Animals are sentient beings, capable of experiencing emotions. Being able to assess emotional states in farm animals is crucial to improving their welfare. Although the function of emotion is not primarily for communication, the outward expression of an emotional state involves changes in posture, vocalisations, odours and facial expressions. These changes can be perceived and used as indicators of emotional state by other animals. Since emotions can be perceived between conspecifics, understanding how emotions are identified and how they can spread within a social group could have a major impact on improving the welfare of farmed species, which are mostly reared in groups. A recently developed method for the evaluation of emotions in animals is based on cognitive biases such as judgment biases, i.e. an individual in a negative emotional state will show pessimistic judgments while an individual in a positive emotional state will show optimistic judgments.

The aims of this project were to (A) establish whether sheep and goats can discriminate between images of faces of familiar conspecifics taken in different positive and negative situations, (B) establish whether sheep and goats perceive the valence (positive or negative) of the emotion expressed by the animal on the image, (C) validate the use of images of faces in cognitive bias studies.

The use of images of faces of conspecifics as emotional stimuli was first validated, using a discrimination task in a two-armed maze. A new methodology was then developed across a series of experiments to assess spontaneous reactions of animals exposed to video clips or to images of faces of familiar conspecifics. Detailed observations of ear postures were used as the main behavioural indicator. Individual characteristics (dominance status within the herd, dominance pairwise relationships and human-animal relationship) were also recorded during preliminary tests and included in the analyses. The impact of a low-mood state on the perception of emotions was assessed in sheep after subjecting half of the animals to unpredictable negative housing conditions and keeping the other half in good standard housing conditions. Sheep were then presented with videos of conspecifics filmed in situations of varying valence. Reactions to ambiguous stimuli were evaluated by presenting goats with images of morphed faces. Goats were also presented with images of faces of familiar conspecifics taken in situations of varying emotional intensity.

Sheep could discriminate images of faces of conspecifics taken either in a negative or in a neutral situation and their learning process of the discrimination task was affected by

the type of emotion displayed. Sheep reacted differently depending on the valence of the video clips ( $P < 0.05$ ); however, there was no difference between the control and the low-mood groups ( $P > 0.05$ ). Goats also showed different behavioural reactions to images of faces photographed in different situations ( $P < 0.05$ ), indicating that they perceived the images as different. Responses to morphed images were not necessarily intermediate to responses to negative and positive images and not gradual either, which poses a major problem to the potential use of facial images in cognitive bias experiments.

Overall, animals were more attentive towards images or videos of conspecifics in negative situations, i.e., presumably, in a negative emotional state. This suggests that sheep and goats are able to perceive the valence of the emotional state. The identity of the individual on the photo also affected the animals' spontaneous reaction to the images. Social relationships such as dominance, but also affinity between the tested and photographed individual seem to influence emotion perception.

# Lay summary

Animals are capable of experiencing emotions. Being able to assess emotions in farm animals is crucial to improving their welfare. A new method for the evaluation of emotions in animals is based on cognitive or judgment biases, i.e. an individual in a negative emotional state will show pessimistic judgments while an individual in a positive emotional state will show optimistic judgments. Expression of emotions involves changes in posture, vocalisations, odours and facial expressions. These changes can be perceived and used as indicators of emotional state by other animals. Since emotions can be perceived by conspecifics, understanding how emotions are identified and how they can spread within a social group could have a major impact on improving the welfare of farmed species, which are mostly reared in groups.

The aims of this project were to (A) establish whether sheep and goats can discriminate between images of faces of familiar conspecifics taken in positive and negative situations, (B) establish whether sheep and goats perceive the valence (positive or negative) of the emotion expressed by the animal on the image, and (C) validate the use of images of faces in cognitive bias studies.

I demonstrated that sheep and goats could discriminate between images of faces of conspecifics taken in different situations (positive v. negative and neutral v. negative). Overall, animals were more attentive towards images or videos of conspecifics in negative situation, i.e. presumably in a negative emotional state, which suggests that sheep and goats are able to perceive the valence of the emotion expressed. The identity of the individual in the photo also affected the animals' spontaneous reaction to the images. Social relationships such as dominance, but also affinity between the tested and photographed individual seem to influence emotion perception. Further studies are needed to reach a definite conclusion on the use of images of faces in cognitive bias methodology.

## Publication

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[based on Chapter 4]

## Conferences contributions

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## List of abbreviations

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<b>AU</b>	Action unit
<b>CI</b>	Confidence interval
<b>EIA</b>	Enzyme immunoassay
<b>FACS</b>	Facial action coding system
<b>FGM</b>	Faecal glucocorticoids
<b>FTS</b>	Final training session
<b>GLMM</b>	Generalised linear mixed model
<b>HAT</b>	Human approach test
<b>HS</b>	Handling score
<b>REML</b>	Restricted maximum likelihood

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# Chapter 1: General Introduction

## 1.1 Introduction

The idea that animals are capable of experiencing negative and positive emotions is now widely accepted both in the scientific community (Panksepp, 1998; Boissy et al., 2007; for a review see de Vere and Kuczaj, 2016) and this definition of animal sentience has been included by policy makers in the Treaty of Lisbon (European Union, 2008). Despite this recognition of animal sentience (as defined by the ability to experience emotions), the current knowledge of animal emotions is still incomplete and improving our understanding of emotions in animals has become one of the major scientific challenges of recent years.

So far, the main focus of research in animal emotions has been the evaluation of emotional states in the individual animal (Spinka, 2012), and the development of welfare assessment tools allowing human observers to recognise emotional states in animals. However, emotions also have a communicative aspect. The expression of emotional states indeed elicits behavioural changes which can be perceived and used as indicators of emotional state by other animals (Shariff and Tracy, 2011). Since these changes can be perceived by conspecifics, it is important to understand how emotions are identified and how they can spread within a social group. Emotional contagion, i.e. the shifting of an animal's emotional state towards the emotional state perceived in a conspecific, is a widely found phenomenon (Edgar et al., 2012). Emotional contagion could have a major impact on the welfare of group-reared farm species since, for instance, one individual in a negative emotional state could potentially have a negative effect on the whole group. This is why the main aim of this PhD project was to investigate the perception of emotions in animals.

When studying emotions, it is first necessary to define more precisely what emotions are. This has been the subject of much controversy, and Section 2 of this chapter will summarise of the current theoretical frameworks available for the study of

emotions. The work presented in this thesis was mostly specifically focused on facial expressions of emotions and their perception. Faces are highly complex stimuli, and an overview of face perception, facial expressions and face-based individual recognition in mammals will be then presented in Sections 3 to 5 of this chapter.

The emotional state of an animal can influence cognitive processes, such as learning, attention or judgement (Mendl et al., 2009). In brief, judgement bias is based on the hypothesis that animals in a negative emotional state will show pessimistic judgements about ambiguous stimuli (i.e., react in a similar way to negative and ambiguous stimuli) while those in a positive emotional state will make optimistic judgements (i.e., react in a similar way to positive and ambiguous stimuli). Face-based perception of emotion is a cognitive process (Martin et al., 2012) and as such is potentially subject to judgement bias too. The second aim of this project was therefore to explore the potential use of faces as cues in judgement bias studies. The judgement bias paradigm as well as its applications and limitations will be presented in details in Section 6 of this chapter. The interest in the use of cognitive bias method for the study of emotion perception will be addressed in the final part of this section (Section 6.4). The final section of this chapter (Section 7) will introduce the details of the objectives and outlines of the experimental work presented in this thesis.

This project focused on sheep and goats. Small ruminants represent an excellent model for the study of emotion perception. Goats and sheep are highly gregarious species that establish complex social relationships (Barroso et al., 2000; Nowak et al., 2008). Sheep and goats also have excellent visual acuity (Blakeman and Friend, 1986; Sugnaseelan et al., 2013) and use visual signals in social communication (Nowak et al., 2008). In both species, behavioural (e.g. ear postures, vocalisations) and physiological (e.g. variations in heart rate) indicators have been associated with positive and negative emotional states (Boissy et al., 2011; Briefer et al., 2015). Furthermore, small ruminants, and more widely the majority of livestock species are

bred and raised in groups. Understanding how the emotions experienced by one individual can be perceived by conspecifics and can potentially influence the emotional state of others in the group is a very important first step in our comprehension of the social dimension of animal welfare. The literature reviewed in this chapter will thus have a particular focus on sheep and goats, but also more widely on farm species.

## 1.2 Defining emotions, an ongoing challenge

First of all, an important clarification needs to be made regarding the vocabulary used in the emotion literature. The term ‘emotion/emotional state’ represents the umbrella term that covers the behavioural, physiological and cognitive changes that occur in a subject while the term ‘affect/affective state’ is defined as the conscious experience of an emotion, the subjective component presented below (Panksepp, 1998; Mendl et al., 2010).

To date, there is no consensus in the human psychology literature on a conceptual framework for emotions or affective states; however, certain similarities emerge between definitions and can thus be used to form an operational definition of emotions. Across the theories presented, it is generally agreed that an emotion is an intense but short-lived affective response to an event, and materialised through specific body changes (Parkinson, 1995; Désiré et al., 2002). An emotion can be characterised by its components: a behavioural component (a posture or an activity), an autonomic component (a physiological response) and a subjective component (emotional response or ‘feeling’) (Dantzer, 2002). More recently, a cognitive component has been added to the description of emotions, relating to the cognitive evaluation of the situation eliciting the emotional state (Lazarus, 1991).

Theories of emotions can be grouped into three main branches: discrete theories, dimensional theories and appraisal theories. First, advocates of discrete theories of emotions support the idea that a few distinct fundamental emotions are

present in all mammals and that these basic emotions can interact to form more complex ones. Basic emotions are also characterised by specific and experimentally established physiological and behavioural changes, which contribute to the individual's response to situations. For instance, Panksepp's model of emotions includes seven 'primal' emotions: PLAY, PANIC, FEAR, RAGE, SEEKING, LUST and CARE (Panksepp, 2005). In this approach, discrete emotions are supported by the activation of specific brain regions and neural circuits (Panksepp et al., 2011). Such circuit models are supported mainly by animal studies and neuroimaging and have helped to demonstrate similarities between the emotions of humans and other mammals (Burgdorf and Panksepp, 2006; Berridge and Kringelbach, 2008). The model of basic emotions presented by Ekman (1999) is based on the universality of emotions that can be identified across cultures in humans (and to a certain extent across species) as shown by similar facial and behavioural expressions that co-occur in specific situations eliciting emotional states. Ekman's model is more detailed than Panksepp's and includes: amusement, anger, contempt, contentment, disgust, embarrassment, excitement, fear, guilt, pride in achievement, relief, sadness, satisfaction, sensory pleasure and shame (Ekman, 1999).

Dimensional theories of emotions are based on verbal self-report of emotional states. Different models have been proposed (Russell, 1980; Plutchik, 1994; Watson et al., 1999; Cabanac, 2002), but they all concur in describing emotions along two axes: a valence dimension (positivity vs. negativity) and an arousal dimension (high vs low activation). Emotions represented in this two-dimensional space are called core affects (Russell, 2003). Discrete emotions, such as fear or anger, can be represented in the core affect space. For instance fear has a negative valence and a high arousal while happiness has a positive valence and a high arousal. Keeping this framework in mind, the term 'neutral state' used in this thesis will refer to a state of very low arousal and of very low positive or negative valence, that could be observed in relaxed situations such



as 'ruminating while lying down' in sheep or 'relaxed and sleepy' in horses (Wathan et al., 2016).

Finally, a third approach to emotions is appraisal theories, which posit that emotions arise from a series of checks (namely, the appraisal) that allow the subject to evaluate a situation (Frijda, 1987; Lazarus, 1991; Scherer, 2009). The different steps of the appraisal cover the evaluation of the intrinsic characteristics of the situation (e.g. pleasant/unpleasant, novelty, suddenness), its significance in terms of needs for the subject, potential coping strategies available to the subject and its significance in terms of social and personal standards. It is through the appraisal of a situation that the cognitive component influences the emotional response of the individual. Moreover, the same situation might be appraised differently by different subjects, depending on previous personal experience or differences in motivations, which may lead to a wide range of emotional states induced by different individual experience of a specific stimulus (Scherer, 2009). Appraisal theories have been successfully translated to the study of emotions in animals in experiments that compared the appraisal processes for a given situation with more classic behavioural and physiological measures (Désiré et al., 2002; Désiré et al., 2004; Greiveldinger et al., 2007).

There is behavioural, psychological and neuroimaging evidence for both the dimensional and discrete theories of emotions, and a co-existence of both models has been proposed (Mendl et al., 2010). The framework proposed by Mendl et al. integrates discrete emotions into the valence and arousal dimensions, and thus accounts for subtle variations in emotional states and for a wide range of emotions (Figure 1.1). This framework also provides a functional approach based on the adaptive value of emotional states. Hence, rewarding or fitness-enhancing stimuli will elicit a shift into positive emotional states, while punishing or fitness-threatening stimuli, such as predators, pain or stress, elicit a shift into negative emotional states (Figure 1.1). Anecdotally, a recent survey of researchers who study (mostly human) emotions

(Ekman, 2016) has revealed that the agreement between researchers on which theoretical frameworks are appropriate for the study of emotions is higher than it was 20 years ago (Ekman and Davidson, 1994). Aspects from both the discrete and the dimensional approaches are now often considered simultaneously (Ekman, 2016).

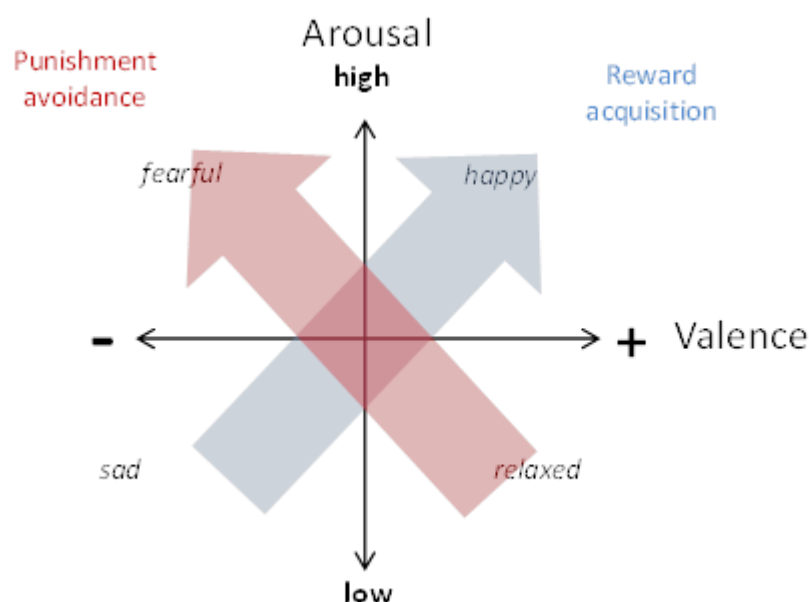


Figure 1.1. **Representation of core affects in the valence and arousal dimensions.** The more positive emotional states are found in the right-hand side quadrants, and the more negative emotional states in the left-hand side quadrants. The two arrows represent the systems based on Mendl et al.'s functional approach: rewarding stimuli elicit a shift into positive emotional states (right-hand side quadrants), while punishing stimuli elicit a shift into negative emotional states (left-hand side quadrants). Adapted from Mendl et al. (2010)

### 1.3 Faces: an essential source of information

The basic configuration of a face is known as the 'first-order features'. In humans, and more generally in mammals, these first order features consist of two eyes placed above a nose, which is placed above a mouth (Diamond and Carey, 1986). This basic arrangement is important for discriminating faces from other items. In human neonates, attraction to these first-order features (two eyes above a nose above a mouth) is already strong (Heron-Delaney et al., 2011). Similar observations have been made in non-human primates, and, there, too, this attraction to faces appears to be innate (reviewed by Parr, 2011). 'Second-order features' consist of all the individual variations around this pattern. External (e.g. hair style, shape of the head, position of

the ears) and internal (e.g. colour of the eyes, the shape of the nose, or the width of the mouth) second order features can be distinguished. Different types of information can be extracted from a face: identity, communication, emotional expression, attraction, gaze direction (Leopold and Rhodes, 2010). In this chapter the focus will be placed on face recognition and facial expressions.

Face recognition is often described as the peak of human visual performance (Leopold and Rhodes, 2010). The information needed to identify an individual is mainly extracted from second-order features (Diamond and Carey, 1986). An essential characteristic of face processing in humans is that the features of a face and their relative spatial arrangement are perceived as a gestalt, i.e. as a whole (Farah et al., 1998). This holistic processing is apparent in face recognition tasks where pictures of modified faces are presented. Subtle differences, such as changes in facial expressions, new viewing angles or ageing, are easily processed and do not impair the identification of the individual (reviewed by Posamentier and Abdi, 2003). The most famous consequence of the human holistic processing of faces is the inversion effect, i.e. the impairment in the recognition of faces when they are upside-down. This inversion effect does not affect the recognition of stimuli other than faces in humans (Diamond and Carey, 1986). The processing of faces is also characterised by a right brain hemisphere bias (Sergent et al., 1992). Humans preferably look at faces with their left eye (the left visual field is associated with processing in the right hemisphere), and faces presented in the left visual field are identified more rapidly and more accurately (Hilliard, 1973; Young and Ellis, 1976; Anzellotti and Caramazza, 2014).

To date, all species of non-human primates that have been tested experimentally are able to recognise familiar individuals from pictures of their faces (for a review, see Parr, 2011). Chimpanzees seem to be the closest to humans in their abilities: they are able to learn to discriminate between pictures of unfamiliar individuals, to identify familial resemblance in unfamiliar faces (Parr and de Waal,

1999), and their ability to recognise faces is affected by the inversion effect and changes in second-order features (Parr et al., 1998a). Monkeys (e.g. rhesus macaques and capuchins) also have the ability to discriminate between pictures of unfamiliar conspecifics and to detect familial similarity in pictures of unfamiliar monkeys. However, they do not show a clear inversion effect, and their processing of facial features seems to be mainly focused on first-order features (Leopold and Rhodes, 2010).

Diurnal social mammals, such as ungulates (e.g. sheep, goats, horses or cattle) also use visual cues for recognition (Tate et al., 2006). Dairy cows are able to discriminate between images of faces of both familiar and unfamiliar individuals from their own breed (Holstein) and from very different breeds such as Charolais (Coulon et al., 2009). So far, the sheep is the most studied non-primate species in terms of face perception and face processing. Mother-offspring recognition, perception of images of conspecifics, and face recognition have been explored in sheep. The use of visual cues to identify other individuals was first demonstrated in mother ewes and lambs in the 1970's. When the heads of lambs were dyed in a darker colour, ewes had increased difficulties recognising their lambs compared to when other parts of the body were dyed or when the lamb was not dyed (Alexander and Shillito, 1977). It has also been shown that sheep perceive pictures of conspecifics as social stimuli and the presence of a projected image of another sheep reduces stress and fear responses in social isolation (Bouissou et al., 1996; da Costa et al., 2004).

Behavioural and neurological studies conducted by Kendrick and his colleagues have shown that sheep possess remarkable face-recognition abilities. Experimental sheep discriminated between sheep and human faces, between different breeds of sheep, between sexes in their own breed, and between individuals in both sheep and humans (Kendrick et al., 1995). Their visual acuity is excellent (Sugnaseelan et al.,

2013) and sheep were also able to discriminate morphed faces of conspecifics differing by only 10% (Tate et al., 2006).

Sheep learned to discriminate between faces of other sheep to obtain a reward faster than they learned to discriminate between faces of humans or between different simple geometric symbols. Learning was also faster with faces of familiar individuals (Kendrick et al., 1996). Once they had learned that the face of a given conspecific was associated with a reward, sheep remembered this information for over two years (Kendrick et al., 2001). Sheep were also able to transfer the discrimination learnt with images of faces of 3-month-old lambs to images of the same lambs but at a younger age (Ferreira et al., 2004). The remarkable abilities of sheep in face perception are most likely attributable to their use of internal second-order features (e.g. distance between the eye, shape of the nose) to identify conspecifics (Peirce et al., 2000). Conversely, in their recognition of human faces, sheep have been shown to rely more on the external second-order features such as hair-style, shape of the face, shape and position of the ears (Peirce et al., 2001). Finally, like humans, sheep have been found to be sensitive to the inversion effect. Discrimination between faces was impaired when images of faces, but not of other objects, were presented upside-down (Kendrick et al., 1996). Sheep also share with humans and non human-primates (monkeys: Guo et al., 2009; chimpanzees: Dahl et al., 2013) a right brain hemisphere/left visual field bias for face recognition that is not present when processing geometric shapes or images of objects (Broad et al., 2000; Kendrick, 2006).

## 1.4 Facial expressions of emotions in humans and animals

In his book entitled *Expression of the Emotions in Man and Animal* (1872), Darwin presented face perception in an evolutionary perspective for the first time. Through precise descriptions of the display of emotions in several species, he suggested that facial expressions had much in common in humans and non-human

mammals and thus facial expressions had similar origins and served similar functions across these species. Facial expressions can be considered as both an emotional response and an element of social communication (Shariff and Tracy, 2011). The evolutionary role of expressions of emotion and their importance as social signals has been demonstrated in laboratory-reared rhesus monkeys that learned to be afraid of snakes after only seeing wild-reared monkeys displaying fear expressions in the presence of snakes (Mineka and Cook, 1993).

Of all non-human mammals, primates can display the widest repertoire of expressions through their highly developed orofacial motor system (Burrows, 2008). This repertoire consists of expressions involving the furrowing of the brow, the upturn or downturn of the mouth, the display of the teeth, the widening of the eyes, and changes in ear postures. Some of these expressions are unique to humans or apes, and some are not. Facial expressions are by nature highly graded and flexible signals (Waller and Micheletta, 2013), and they can vary in intensity or between individuals. This is why obtaining high quality standardised stimuli for experimental studies of animal or human emotions can be challenging. The Facial-Action Coding System (FACS) that was developed by Ekman and Friesen (1978) for the study of humans has been an important tool for quantifying variation in the intensity and the shape of facial expressions and for allowing the clear categorisation and standardisation of facial expressions. FACS is based on the anatomy of the face and on the movements of the facial muscles. FACS breaks down facial expressions into Action Units (AU), each corresponding to the contraction of a specific muscle. FACS allows facial expressions to be described in a standardised way, and similarities in expressions to be identified across emotional situations and cultures. For instance an expression of fear would be described as the following combinations of AU: AU 1 (inner brow raiser) + 2 (outer brow raiser) + 23 (lip tightener) + 26 (jaw drop). FACS has also been adapted to study non-human species, starting with chimpanzees (Vick et al., 2007), and then progressing

to macaques (Parr et al., 2010), gibbons (Waller et al., 2012), and orang-utans (Caeiro et al., 2013b). Tools such as FACS have allowed great progress to be made in the study of facial expressions in animals.

Originally, a complex facial musculature, such as that found in primates, was deemed essential to display facial expressions. More recently, however years, the study of facial expressions of emotions in other species has brought to light the existence of rich repertoires of facial expressions across mammals. For instance, the response to the taste of food has been a useful tool in the evaluation of facial expressions in animals. Facial expressions can indeed vary in reaction to the hedonic valence of food, i.e. whether it is pleasurable or not (Berridge, 2000). Grill and Norgren (1978) were the first to describe rats' reactions, specifically their mouth and lips movements, to sweet and bitter tastes. However, as is often the case in animal behaviour science, the first evidence of standardised facial expression of emotions came from the study of negative experiences and, more specifically, of pain. Grimace scales are standardised coding systems of facial expressions and were originally used to assess pain in infants or patients where verbal communication is not possible (due to intubation or sedation for instance). Grimace scales have been developed successfully in mice (Langford et al., 2010), rats (Sotocina et al., 2011), rabbits (Keating et al., 2012), cats (Holden et al., 2014), sheep (McLennan et al., 2016), pigs (Di Giminiani et al., 2016), dairy cows (Gleerup et al., 2015) and horses (Dalla Costa et al., 2014). Interestingly, a common set of indicators, such as orbital tightening, backward ear postures or strained jaw muscles, has been identified across these species (Di Giminiani et al., 2016).

Research in facial expressions has recently explored positive emotional states and potential facial indicators of positive emotions. In rats, for example, manual tickling by an experimenter that mimics 'rough-and-tumble play' in juveniles has been found to induce the same ultrasonic vocalisations that are emitted during conspecific play (Panksepp and Burgdorf, 2003). Manual tickling has also been found to be

associated with optimistic judgement bias (Rygula et al., 2012), which suggest that tickling is most likely linked with positive emotional states. Relaxed ear postures and pinker ears have recently been associated with this pleasurable tickling (Finlayson et al., 2016). Cats presented with pleasant food displayed more tongue protrusions, mouth smacks and nose licks, while half-closing their eyes (Hanson et al., 2016). In dairy cows, studies have investigated potential facial indicators such as visible eye-white percentage (Proctor and Carder, 2015) and ear postures (Proctor and Carder, 2014) for positive emotional states. The development of FACS for non-primates species has also been a major advance in the study of the expression of positive emotions that are often associated with more subtle displays. There are now FACS available for horses (Wathan et al., 2015), dogs (Waller et al., 2013) and cats (Caeiro et al., 2013a). We hope to see versions developed for more species, including farm animals, in the future.

## 1.5 Perception of emotions

### 1.5.1 Face-based emotion perception

Facial expressions can convey essential information to conspecifics, and there has been growing evidence for face-based emotion recognition in non-human animals (Leopold and Rhodes, 2010). Amongst other examples, it has been shown that chimpanzees can discriminate between emotional displays from pictures of conspecifics (Parr et al., 1998b). Moreover, ‘matching to meaning’ studies suggest that the valence of the emotion displayed is also perceived, i.e. a positive face (play face) is perceived as positive while a negative face (scream) is perceived as negative (Parr, 2003). In these tasks, chimpanzees had to select one of two facial expressions (play face for positive and scream face or bare-teeth display for negative) that were paired with other facial expressions such as relaxed-lip face) that would match the valence of short video clips they had previously seen (Parr, 2001). To this day, similar matching to



meaning studies have not been done in non-primate species. Facial expressions of emotions are important social signals (Frith, 2009). They are at the heart of social interactions and group cohesion, and non-human primates can adjust and use facial expressions to communicate with conspecifics (Parr et al., 2005; Scheider et al., 2016). A clear example is the use of facial displays of aggression to limit actual aggressive interactions: threatening and submissive facial expressions can allow animals to avoid energy-consuming, risky antagonist interactions, and to resolve conflicts easily (Judge and de Waal, 1993; Otovic et al., 2014).

In farm species, sheep typically prefer familiar faces; however, when sheep are simultaneously presented with the stressed face of a familiar conspecific and the calm face of an unknown sheep, they demonstrate a preference for the calm face (Tate et al., 2006). A recent study also showed that horses can discriminate between faces of conspecifics displaying either positive attention or relaxed neutral posture and faces displaying agonistic expressions (Wathan et al., 2016). The interspecific discrimination of emotions has also been demonstrated in domestic species that interact with humans. for instance, dogs (Albuquerque et al., 2016), sheep (Tate et al., 2006) and horses (Smith et al., 2016) discriminate not only between calm and stressed faces of conspecifics, but also between these expressions in humans.

### 1.5.2 Variability in emotion perception

Responses to emotional stimuli, including facial expressions, can vary considerably between individuals. Several factors have been identified as accounting for this variability, and these factors have mostly been related to differences in brain processing (Hamann and Canli, 2004). First, personality traits can influence the perception of emotions (Calder et al., 2011). The Five-Factor Model of personality includes openness, agreeableness, conscientiousness, extraversion and neuroticism (Digman, 1990). Neuroimaging studies in humans have established links between personality traits and individual differences in the level of activation of certain brain

areas when presented with stimuli of varying valence. For instance, Canli et al. (2002) found a positive correlation between participant's extraversion and activity levels in the left amygdala when participants were presented with happy faces. Similarly, variation in response to fearful faces has been associated with variation in non-clinical anxiety levels in subjects (see Bar-Haim et al., 2007, for a meta-analysis).

In humans, empathy and face-based emotion recognition influence each other, which once again highlights the importance of faces in social interactions. The ability to correctly identify the emotion conveyed by a facial expression is necessary for any empathetic response. However the degree of empathy of the subject looking at the expression can also explain some of the observed variation in the perception of facial expression. It has indeed been shown that more empathetic subjects have better face-based emotion recognition abilities. For instance, subjects with a higher self-reported measures of empathy took less time to identify correctly the emotions presented on images of human faces (Martin et al., 1996; Besel and Yuille, 2010). In a recent study, the influence of the degree of empathy on perception of dog faces by humans was assessed. More empathetic subjects (as assessed on the Interpersonal Reactivity Index (Davis, 1980)) identified the valence of dogs facial expressions faster and evaluated threatening faces in dogs as more negative (Kujala et al., 2017). There is also evidence for empathy in non-human animals (de Waal, 2007). For instance, apes and corvids display consolation behaviour after conflicts that are not displayed in control situations (embracing in chimpanzees, Kutsukake and Castles, 2004; interlocking beaks, synchronised bowing and tail fanning in rooks, Seed et al., 2007). In pairs of mice exposed to painful stimuli, the pain behaviour of one individual intensified or decreased depending on whether the second mouse was in pain too (Langford et al., 2006). Nonetheless, no direct link between face-based emotion recognition abilities and empathy levels has been established in mammals as yet.

Sex is also an important factor that affects face-based emotion perception, and differences in brain activity between men and women have been found in responses to happy and sad facial expressions (Lee et al., 2002). For instance, men showed a higher activation in the left hemisphere when looking at sad faces compared to happy faces, but in women brain activation did not differ between the two types of faces (West et al., 2001). Finally, individual differences in face-based perception of emotion have also been linked to the genotype. Briefly, a combination of two alleles from genes coding for the catecholamine system, has been found to be associated with faster emotion recognition in men and with differences in perception of valence and arousal (Tamm et al., 2016).

In this section, I have only examined the stable factors that can affect emotion perception (personality, sex, and genotype) and which should be taken into account when designing studies on emotion perception in animals. The effect of the emotional state of the subjects on their perception of emotions deserves an entire section and will be addressed in more detail later in this chapter.

## 1.6 Measuring emotions in animals

### 1.6.1 Faecal glucocorticoids

In animals, the subjective component of an emotional state can only be estimated via changes in its behavioural and physiological components. Glucocorticoids are a key component of the endocrine mechanisms involved in responses to stressful situations. Glucocorticoids are produced by the adrenal glands, which are central in the reactions to stress as they are involved in the hypothalamic–pituitary–adrenocortical (HPA) axis and the sympatho-adrenomedullary system (Möstl and Palme, 2002). When a stressful situation occurs, the adrenal glands are triggered by the activation of the HPA axis in response to the stressor, leading to an increase in the glucocorticoids secretion. Glucocorticoids help to increase energy mobilisation for

the response to short-term stressors (Korte et al., 1993). It is also important to point out that glucocorticoid can be released during situations that are not per se negative for the individuals, such as during sexual behaviour, birth or hunting (Palme, 2012). The measurement of glucocorticoids has traditionally been used as a way to evaluate stress and pain in animals (Stubsj  en et al., 2015). Depending on the species studied, the type of glucocorticoid measured varies (e.g. corticosterone in rats and cortisol in ruminants (Palme, 2012). Because they are easier to collect than blood samples, faecal samples have been particularly used to measure glucocorticoids concentrations. In these faecal samples, the concentrations measured represent the cumulative secretion of hormones over a given period of time, and are less affected by short-term variations (such as can be caused by handling stress) than blood levels for example (Palme, 2012). The quantification of faecal glucocorticoids metabolites (FGM) has been validated in sheep (Palme and M  stl, 1997) and goats (Kleinsasser et al., 2010).

However, FGM results should always be interpreted with caution, as responses to stressors are highly context-dependent. Regarding the use of FGM to quantify chronic stress in sheep, as induced by exposure to negative housing conditions, results for FGM can differ between studies. For instance, after 9 weeks of exposure to unpredictable negative housing conditions, no differences were found in baseline blood cortisol levels between treatment and control group in sheep (Destrez et al., 2013b). In another sheep study involving 4 weeks of negative housing conditions, blood cortisol levels decreased within the treatment group, while there was no difference before or after the treatment between control and negative housing conditions group (Doyle et al., 2011). This is why the use of a combination of behavioural and physiological measurements is recommended (Palme et al., 2012).

### 1.6.2 Ear postures

The validation of behavioural indicators to evaluate the emotional states of individual animals has been a major focus of applied ethology. Behavioural indicators

indeed allow the experimenters to avoid the stressful handling that is often required for sampling of physiological indicators. It also makes it possible to observe animals from a distance and within their social group. In ruminants, ear postures have received a lot of attention (Reefmann et al., 2009a; Proctor and Carder, 2014; Briefer et al., 2015). Studies in sheep and goats have aimed to first identify which ear postures are effectively used by the animals (Boissy et al., 2011), then to correlate these ear postures with other behavioural and physiological measurements of emotional states in experimental situations (Briefer et al., 2015).

The analysis and interpretation of ear postures results should always be done cautiously. Indeed, different situations can induce similar emotional states and ear postures. For instance, social isolation (Briefer et al., 2015) and pain caused by castration and tail docking (Guesgen et al., 2016), both negative situations, have been associated with backward ear postures in small ruminants. However, in sheep, lower percentages of ears backwards have been observed when animals were exposed to a physical stimulus of negative valence (pricking) than to a positive stimulus (kneading) (Vögeli et al., 2014b). Similarly, a higher proportion of backward ear postures was observed during pleasurable grooming in dairy cows (Proctor and Carder, 2014). The meaning of backward ear postures thus seems to be context-dependent, and more study is needed to determine if backward ear postures can be more definitely associated with the valence of a situation.

In sheep and goats, a higher percentage of time spent with the ears forward has been observed in situations with a negative valence, such as when the animal is being pricked by an experimental device (Vögeli et al., 2014b) or when the animal is in socially isolated (sheep: Reefmann et al., 2009c; goats: Briefer et al., 2015). However, a decrease in the percentage of time spent with the ears forward was also observed after tail-docking and castration in lambs (Guesgen et al., 2016), suggesting that the association between ears forward cannot be generalised to all negatively valenced

situations. In fact, forward ear postures have also been observed in situations where a high level of attention is required, i.e. eliciting high arousal (exposure to an unfamiliar test situation involving mild pain in sheep (Stubsjøen et al., 2009), or novel odour test in wild mice (Lecorps and Féron, 2015)). Situations eliciting high arousal often coincide with a negative valence, but empirical observations have also identified forward ear postures in what could be considered positive situations, for instance, while the animals approached rapidly a bucket containing food pellets or when a familiar human entered the barn (personal observations). A higher percentage of time spent with the ears forward could then be associated with situations that lead to high arousal and/or increased attention, rather than to negative situations *per se*.

Finally, in the majority of studies, ear postures are observed and analysed with a sum-one constraint and are thus not independent (Guesgen et al., 2016). In other words, if the proportion of time spent with the ears forward decreases, the proportion of time spent in other ear postures increases. Changes in ear postures should thus be interpreted simultaneously and it is not the changes in ear postures, but rather the direction of the change (higher proportion of ears forward for instance) that is of interest.

## 1.7 Cognitive bias

### 1.7.1 Judgement bias paradigm

Cognitive functions refer to the information processes that include attention, learning, memory and decision making (Shettleworth, 2010). Human psychology has established that cognitive processes affect and are affected by the valence of an individual's emotional state. For instance, people in a negatively valenced affective state (i.e. anxiety, depression, or an experimentally induced emotional state) pay more attention to threatening stimuli than people in a neutral or positive state (Austin et al., 2001; Gotlib et al., 2004). Anxious patients also interpret ambiguous stimuli negatively,

for example when asked to spell homophones, such as “dye”/“die”, they choose the threatening one, i.e. “die” displaying a negative judgment bias (reviewed in Paul et al, 2005).

In humans, the subjective component of an emotional state is typically measured via linguistic self-report. In animals, this subjective component can only be estimated via its behavioural and physiological components. As such, classic methods for investigating animal emotions use the measurement of physiological (e.g. heart rate, corticoids, surface temperature...) and behavioural (e.g. locomotion, vocalisations, play behaviour) indicators of stress and reduced well-being. However, these measures may be better at detecting the arousal rather than the valence of an emotional state (Mendl et al., 2009) since changes in the level of activation are more likely to affect physiological and behavioural characteristics. From an animal welfare point of view, valence is the key measure (Reefmann et al., 2012), since good welfare is not only the absence of negative emotional states, but also, and perhaps mainly, the presence of positive ones (Boissy et al., 2007). Hence the cognitive dimension of emotions, and more specifically changes in cognitive processes leading to cognitive biases, could be as reliable indicators of emotional states in animals as they are in humans (Paul et al., 2005).

Over the last ten years, cognitive bias theory has been extended to non-human animals with varying success, and the potential weaknesses of this method are detailed in Section 1.6.3 of this chapter. Most studies carried out so far on cognitive biases in animals have considered judgement biases, i.e. the propensity of a subject to show a behaviour indicating anticipation of either relatively positive (‘optimistic’) or relatively negative (‘pessimistic’) outcomes in response to affectively ambiguous stimuli (Mendl et al., 2009). Those biases in judgement are likely to be the result of affective influences on the cognitive processes mentioned above.

The judgement bias paradigm was first introduced in rats (Harding et al., 2004). In this original trial, rats were trained to press a lever when presented with a given cue (a tone of a certain frequency) to experience a positive event (food reward) and to refrain from pressing the lever when presented with a second cue (a tone of a specific different frequency) to avoid a negative event (a burst of white noise). To induce differences in affective states, half of the rats were then housed under unpredictable conditions for seven weeks, a treatment which is believed to induce chronic stress and a depression-like state characterised by anhedonia, and enhanced reaction to fear-eliciting events (Zurita et al., 2000) . After this affect manipulation phase, all rats were finally tested by exposing them to ambiguous probe cues (tones of an intermediate frequency). Harding et al. hypothesised that rats from the unpredictable housing conditions would be more likely to consider the ambiguous cues as predicting the negative event (pessimistic bias) and thus refrain from pressing the lever, and this is indeed what they found.

Since this initial study, similar judgement bias studies, with some improvements and modifications to the experimental design, have been used in a range of mammals but also in insects and fish. A non-exhaustive list would include rats (Burman et al., 2009), birds (quail: Horváth et al., 2016; canaries: Lalot et al., 2017), rhesus macaques (Bethell et al., 2012), dolphins (Clegg et al., 2017), dogs (Burman et al., 2011), pigs (Scollo et al., 2014; Dupjan et al., 2016), dairy calves (Daros et al., 2014), goats (Baciadonna et al., 2016) and sheep (Doyle et al., 2010a; Verbeek et al., 2014a), but also bees (Perry et al., 2016), ants (d'Ettorre et al., 2016), zebrafish (Wojtas et al., 2015) and squid (Takeshita and Sato, 2016). The majority of these studies focused on negative emotional states and negative judgment biases, but a few examples of positive judgement biases can also be found (Matheson et al., 2008; Doyle et al., 2010a; Burman et al., 2011; Brydges et al., 2011; Rygula et al., 2012). Not all studies were able to identify judgement biases and to replicated findings similar to Harding et al. (Brilot et



al., 2009). Nonetheless, the wide array of species used in judgement bias studies provides good evidence of the link between emotional states and cognitive processes and of the external validity of judgement bias testing as a method to access emotional states.

### 1.7.2 Applications

Judgement bias method has been applied to the evaluation of animal welfare. Most types of affect manipulation used in these studies were relevant to welfare issues in farm or laboratory species and involved hunger (Verbeek et al., 2014a), social isolation, environmental enrichment (Douglas et al., 2012) and housing conditions (Barker et al., 2016). Studies also investigated the impact of husbandry practices such as shearing (Sanger et al., 2011) or disbudding (Neave et al., 2013). Baciadonna and McElligott (2015) reviewed the use of cognitive bias method as a welfare assessment tool. They concluded in favour of the use of cognitive bias to assess negative emotional states, since in livestock species, studies involving long-term stressors (Doyle et al., 2011), psychological stress (Daros et al., 2014), painful husbandry procedures (Neave et al., 2013) or pharmaceutical treatments (Verbeek et al., 2014b) had been able to detect negative judgement biases. They also concluded that the evaluation of positive emotional states and the detection of 'optimistic' judgement bias were not sufficiently validated yet as too little studies had been carried out with the original aim of identifying positive judgement biases. This weaker argument for the evaluation of positive states could be due to the current limited knowledge of positive emotional states and positive judgement bias ensuing from the lack of investigation in this area, as highlighted above.

Studies comparing anxiety-like states and depression-like states in male chicks showed that anxiety- and depression-like states increased 'pessimistic' responses while only depression-like states decreased 'optimistic' responses. Anxiety was associated with an increased expectation of negative events while depression induced

a decreased expectation of positive events (Salmeto et al., 2011; Hymel and Sufka, 2012). This is similar to findings in humans where anxious and depressed patients exhibit different types of biases (Miranda and Mennin, 2007). Hence, the type of judgement bias observed seems to depend not only on the valence of the underlying emotional state, but more precisely on the type of emotional state. This characteristic of judgment bias makes it a very interesting tool for studies of mood disorders (anxiety and depression) in animal models (Kloke et al., 2014). Cognitive bias has indeed been used in biomedical research, especially to evaluate the effect of anxiolytic drugs (Enkel et al., 2009; Destrez et al., 2012; Verbeek et al., 2014a)

### 1.7.3 Issues with judgement bias method

Even though the study of judgement bias has been successful in assessing animals' emotional states, the judgement bias method is not without issues. Problems emerging from judgement bias studies have been extensively reviewed by Roelofs et al. (2016), and two points are briefly presented here: the discrimination training and the nature of the cues used.

First, judgement bias experiments require an extensive training phase. Animals have to learn to perform the task and to correctly associate the positive and negative cues with their respective consequences. In the majority of studies, animals that could not reach the learning criterion were excluded from the rest of the study, meaning that judgement bias studies are biased towards animals that learned. This extensive training period can also constitute a practical drawback to the use of judgement bias tests to assess welfare, especially in farm animals. It is too time consuming and cannot easily be integrated in on-farm assessment protocols. In the case of studies evaluating the impact of negative situations (e.g. poor housing conditions), it is worth noting that training could also act as cognitive enrichment (Zebunke et al., 2011; Oesterwind et al., 2016) and thus improve the affective state of the animals (Svendsen et al., 2012). Hence, developing judgement bias tasks involving less or no training would improve

both the generalisation of the results (no more 'learners only' bias) and the precision of the results.

Second, a wide variety of cues have been used in judgement bias studies: spatial (location of a feed bucket), auditory (tones of different frequencies), visual (colours), and olfactory (odours). These cues are not all biologically relevant, and do not have an intrinsic value, which is why the discrimination training phase is necessary. Animals thus have to learn to associate one given cue with its consequence to attribute a value to the cue. Using cues that are already considered negative or positive by the animals could be a new way of approaching the judgement bias method. If the intrinsic value of the cues is perceived by the animals without training, it could potentially solve the issues raised by the discrimination task training. The importance of faces as a source of information has been highlighted in this review, and images of facial expressions would potentially represent good candidates for such intrinsically valenced cues.

#### 1.7.4 Cognitive bias in emotion perception

The influence of stable traits (personality, sex or genotype) on emotion perception was presented above, but mood and short-term emotional states can also affect the perception of emotion. Emotion perception is a cognitive process, and as such it is susceptible to the influence of the emotional state of the subject and thus to cognitive bias in the same way that attention or decision-making are. There is indeed evidence from human psychological studies that a negative emotional state such as depression can reduce the ability of the affected individuals to correctly interpret the emotional state of others. Several studies found evidence of a negative judgment bias in emotion recognition in depressed people (Rubinow and Post, 1992; Hale, 1998; Lee et al., 2008b). For instance, depressed patients mistake neutral faces for sad faces (Leppänen et al., 2004). Adults with anxiety disorders also present significant impairment in face-based emotion recognition (see meta-analysis in Demenescu et al., 2010) and anxious adults tend to classify neutral faces as angry. However, a better

recognition of fearful faces by anxious patients has also been reported (Fox, 2002), suggesting an increased attention towards threatening stimuli (Surcinelli and Codispoti, 2006).

Despite the demonstrated importance of faces as social stimuli in animals, there has been little exploration of the impact of an individual's emotional states on their perception of faces and facial expressions. From an animal welfare point of view, if animals in a negative emotional state have an altered perception of social emotional stimuli, it may compromise their ability to integrate into a social group, establish social bonds or avoid aggression or painful situations. On the other hand, animals in a positive mood might be able to better cope with short-term negative events (Reefmann et al., 2012) and provide social support (buffering) to flock members (Rault, 2012). This demonstrates why investigating cognitive biases in the perception of emotional stimuli in farm animals is an important step in our understanding of animal emotions.

To conclude, this review has highlighted the gap that exists between current knowledge of emotions and emotion perceptions in human and non-human primates and in other mammals, especially farm animals. The importance of emotions from an animal welfare point of view has been made very clear by previous research. In farm species, the assessment of emotions in individual animals has been the focus of many studies, but a finer and broader comprehension of how emotions are perceived by others is now needed.

## 1.8 Thesis outline and main objectives

The expression of emotional states can involve changes that can be perceived and used as indicators of emotional state by other animals (Spinka, 2012). Since emotions can be perceived between conspecifics, it is fundamentally important to understand how emotions are identified and how they can spread within a social group. For instance, knowing how negative emotions can affect an entire group and not

only individual animals would provide helpful information to improve management and handling practices of group-reared farm ruminants. Previous research has highlighted the importance of faces as social stimuli, and especially facial expressions. Being able to use images of faces as intrinsically valenced cues in cognitive bias studies could allow us to overcome some of the limitations of the method described in Section 6 of this chapter.

The work presented in this thesis was conducted in order to improve our understanding of emotion perception in small ruminants, and to investigate whether images of faces could be used in judgment bias studies. Two different species were used during this project, sheep and goats. This was originally due to external constraints in animal availability at both experimental farms. Sheep and goats are closely-related species that are similar in their biology and behaviour, even though they also show distinct characteristics (Collias, 1956). However, since the work presented in the thesis focuses on inter-individuals interactions within the same species, the similarities between sheep and goats (complex social structures, excellent visual acuity) were considered strong enough. The differences, especially in behaviour and human-animal relationship were taken into account in the development of the experimental work.

In **Chapter 2**, the use of images of faces of conspecifics as emotional stimuli in sheep is validated, using a discrimination task in a two-armed maze. A new method, developed across a series of experiments to assess spontaneous reactions of animals exposed to video clips or to images of faces of familiar conspecifics, is then described in the following chapters. In **Chapter 3**, the impact of an induced low-mood state on the perception of emotions is assessed in sheep. Half of the animals were subjected to unpredictable negative housing conditions known to induce depression-like states in animals. Sheep were then presented with videos of conspecifics filmed in situations of varying valence. The potential use of images of faces as cues in cognitive bias studies is

assessed more specifically in **Chapter 4**, which describes the spontaneous reactions of goats to morphed images of faces of conspecifics. Results from **Chapter 4** led to reconsider the feasibility of cognitive bias studies with images of faces, and **Chapter 5** investigates at a more fine-grained level of detail the perception of images of faces taken in situations of varying valence and arousal in goats. The study also considered the impact of individual characteristics such as dominance status and human-animal relationship on emotion perception. Finally, the main findings from this PhD project are discussed in **Chapter 6**, as well the implications of these findings for future research.

## Chapter 2: Facial cues influence sheep learning in a visual discrimination task

## Abstract

Faces are an essential source of information for social species. We investigated how sheep use images of faces that were taken in situations of varying valence as cues in a simultaneous discrimination task, and whether the valence of the situation affected sheep performance. To that end, we took photos of faces of sheep in three situations that induced emotional states of neutral (ruminating) or negative valence (social isolation or an aggressive interaction). In a two-armed maze, 35 sheep were then presented with pairs of cues. Sheep had to learn to associate one cue from a pair with a food reward, and the other cue with a punishment (social isolation and unavailable food). Sheep learnt the discrimination with simple coloured cards first, then with pairs of images of the same familiar individual taken in the neutral situation and one of the negative situations. Once they reached the learning criterion with images of faces, sheep had to generalise the task to new pairs of images of different conspecifics. For every run in the maze, the latency to choose an arm and the outcome of the choice (success or error) were recorded, as well as the number of runs needed to learn the task (learning speed). Data was analysed by linear mixed models and learning speed was analysed by Mood's median test. The 16 sheep that learned the discrimination task with coloured cards reached the learning criterion with images of faces. Sheep that had to associate a negative image with a reward learned faster than sheep that had to associate a neutral image with a reward (40 vs. 75 runs,  $\chi^2 = 4.00$ ,  $df = 1$ ,  $P = 0.046$ ). With the exception of sheep from the Aggression-rewarded group, sheep generalised this discrimination to images of new faces ( $F_{3,26.6} = 3.37$ ,  $P = 0.033$ ). Sheep chose an arm correctly more often ( $F_{1,288.1} = 5.02$ ,  $P = 0.026$ ) and more quickly ( $F_{1,289.3} = 23.92$ ,  $P < 0.001$ ; right:  $8.4 \pm 0.6$  sec, left:  $9.6 \pm 0.8$  sec) when the rewarded image was displayed on the right side, suggesting the influence of a right hemisphere/left visual field bias in face-based perception of emotions. Our results indicate that sheep can perceive the emotional valence displayed on faces and that this valence affects learning.



## 2.1 Introduction

Faces are an essential source of information, for social species, from primates to ungulates such as sheep. By looking at the face of another animal, individuals can obtain information about identity, emotional state, sexual attraction or gaze direction (Leopold and Rhodes, 2010). Sheep are one of the most studied livestock species in terms of face processing and are able to discriminate between faces of at least to 50 conspecifics (Kendrick et al., 2001), and to remember these faces for up to two years (Kendrick et al., 2001). Sheep, like cattle, are also sensitive to social familiarity in faces, and show preferences for familiar faces over unfamiliar ones (Tate et al., 2006; Coulon et al., 2011). Individual recognition based on faces also appears to be stable over time in sheep; ewes trained to identify images of faces of three-month-old lambs were then able to discriminate the same lambs aged only one month (Ferreira et al., 2004).

In animals, emotional states can be expressed through vocalisations (Dupjan et al., 2008), odours (Terlouw et al., 1998), posture (Siniscalchi et al., 2013) or facial expressions (Waller and Micheletta, 2013). Outward expressions of emotions are a way of communicating social information to conspecifics as well as across species, as highlighted in recent studies of perception of human faces by dogs and horses (Racca et al., 2012; Smith et al., 2016). Despite their relative lack of facial mobility or of a facial musculature as complex as that of non-human primates, sheep display emotional expressions through their faces, and especially through ear postures (Reefmann et al., 2009a; Boissy et al., 2011). The role of other facial features such as eyes, mouth and cheek muscles have also been identified in facial expressions linked to pain in sheep (McLennan et al., 2016) and lamb (Guesgen et al.). Moreover, conspecifics can distinguish between facial displays of emotions. Indeed, when presented with images of the face of the same familiar conspecific taken in a stressful (isolation) or in a calm situation, sheep showed a preference for the calm face (Tate et al., 2006).

Assessing animals' emotional states has been one of the main focus of animal welfare science, moving from more traditional physiological and behavioural measures to the development of the cognitive bias methodology (Harding et al., 2004). Cognitive bias refers to the influence that the valence of emotional states has on cognitive processes, leading to biases in judgment or attention. For instance, animals in negative emotional states make 'pessimistic' judgements, while animals in positive emotional states show 'optimistic' judgments. This method has been applied to several species, and has especially been used to assess the impact of husbandry practices on the welfare of farmed species (reviewed in Baciadonna and McElligott, 2015). Most recently, judgment bias methodology has even been extended to insects (Perry et al., 2016; d'Ettorre et al., 2016).

The present study was part of a larger project investigating the potential use of images of faces as cues in judgement bias tests with small ruminants. Its first aim was to assess the ability of sheep to distinguish between facial displays of different emotional states. To that end, we first investigated whether sheep could learn to use images of faces of familiar conspecifics displaying different emotional states as cues in a simultaneous discrimination task. We took photos of sheep in three social situations (social isolation, aggressive interaction and ruminating in the home pen) that induced emotional states of different valence. The first phase of training used simple coloured cards, to ensure sheep could learn the discrimination task in the experimental set-up. Sheep were then presented with pairs of images of the same individual but in two different situations.

An important part of cognitive bias studies consist of the training phase, when animals learn to associate one cue with a positive consequence and another cue with a negative consequence. If the valence of the emotional state experienced by a sheep is perceived on images of its face, then faces would be stimuli with an inherent value, and the training phase would not be necessary. Hence, the second aim of this study was to

determine whether sheep could perceive the valence of the emotional state displayed on the image. Social familiarity has been shown to influence learning speed in discrimination tasks, with sheep learning to discriminate faster between faces of a familiar breed than between faces of an unfamiliar breed or symbols (Kendrick et al., 1996). However, little is known about the influence of facial expressions of different emotional states on the learning process in a discrimination task. We hypothesised that learning speed would be affected by the type of images rewarded, i.e. that the valence displayed in the image of a face would affect learning. Presenting pictures of faces has been shown to reduce stress in sheep (Vandenheede and Bouissou, 1994) and images of conspecifics are primary reinforcers, i.e. they are naturally approached by sheep (da Costa et al., 2004). We thus predicted that learning the association between an image of a neutral face, that would be naturally attractive and approached, and a reward would be easier than the association between the image of a stressed face and a reward. We also predicted that this would extend to the generalisation of this association to new images of faces.

Sheep share with humans a left visual field/right hemisphere bias for face recognition that is not present in the processing of images of objects or shapes (Peirce et al., 2000; Kendrick, 2006). Sheep preferentially look at faces with their left eye and display lateralised behaviour when presented with faces (Versace et al., 2007). We were also interested in the potential link between our results and established brain asymmetries in faces and the processing of emotional information. We wanted to test whether sheep would display any side biases corresponding with this left visual field/right hemisphere bias as this would indeed support our hypothesis that sheep did perceive images of faces as faces.

## 2.2 Material and methods

### 2.2.1 Ethical note

All experimental procedures were approved by the SRUC Edinburgh Animal Ethics Committee (ED-AE-2-2014). Animals were weighed weekly before, during and after the study, and were subjected to regular veterinary examinations as part of the farm schedule. No animals had to be removed from the study due to illness or injury. (One sheep had to be excluded because it became apparent after shearing that it was a castrated male and not a ewe.)

### 2.2.2 Animals and housing

Testing took place between March and July 2014 at the SRUC Woodhouselee experimental farm at Easter Bush (UK). Forty non-pregnant female Scottish Mule sheep of 10 to 12 months of age ( $37.1 \pm 4.8$  kg) were used in this study. The sheep were born and reared on the experimental farm and were familiar with each other, having lived in the same flock for at least six months prior to the study. Four sheep (thereafter referred to as Photo Sheep) were pseudo-randomly selected based on body weight so that their body weight was similar to the average body weight of the group ( $37.55 \pm 3.6$  kg). The positive correlation between live weight and hierarchy is well established in ungulates (Drickamer et al., 1999; Landaeta-Hernández et al., 2013) and choosing sheep of intermediate weight was done to avoid the selection of only dominant animals. The Photo Sheep did not take part in the discrimination task but were housed with the rest of the group until the end of the study.

All sheep were housed together indoors in a straw-bedded pen (4 x 12 m) for the duration of the experiment (1.2 m<sup>2</sup> per ewe). They were fed concentrate pellets (0.5 kg per animal per day) and had *ad libitum* access to hay and water.

### 2.2.3 Habituation to handling

Scottish Mules sheep are a hill breed, and typically have very limited contact with humans throughout the year. The experimental animals had little experience of human handling and living indoors, and therefore underwent a short phase of systematic desensitisation to facilitate handling (Hargreaves and Hutson, 1990), and to limit the impact of handling stress on the response to tests. This habituation procedure involved four consecutive steps that allowed the animals to gradually adapt.

This phase of systematic desensitisation took place over three days for all animals. First the animals were handled in two groups of 13 and one group of 14 sheep. Animals was only moved to the next step once all sheep went calmly through the previous step. For the first step, the group was moved into a small handling pen with the gate open. For the second step, the group was confined in a small handling pen with no human handler present. On the second day, the third step consisted of confining the group in the same small handling pen, but with an experimenter standing just outside the pen. Finally for the fourth step, the experimenter had to touch calmly every sheep within the group. Following the fourth step, on the third day, each group of sheep was moved through raceways into 4 x 4 m pen that served as the test arena. Once in the test arena, they were held for ten minutes and received a small amount of concentrate feed. This manipulation was repeated three times with the group size decreasing to five and then to two sheep.

### 2.2.4 Images of faces

We filmed each of the four Photo Sheep in three different situations. For each situation, short video clips of the Photo Sheep were taken with a HD camcorder (Legria HFM52, Canon, Tokyo, Japan). After review of the video clips collected, the most representative facial expression observed during the situation was identified. This consisted of a facial expression consistently presented by all individuals filmed during

the situation. The facial expression also had to occur during the same time-window for all individuals (e.g., within 50 sec of the beginning of the isolation). The selection of this representative facial expression was also based on the general behaviour expressed by the animal, e.g., during increased locomotion for the social isolation. We chose to use a frontal view of the faces, as it was the best way to show the maximum of facial features at the same time to the sheep observing the images (e.g. both ears and eyes visible at the same time). A frontal view of the animal face displaying the most representative facial expression observed during the situation, as detailed below for each situation, was thus extracted from the video clips using Pinnacle Studio 17 (Pinnacle Systems, 2013). Then, using Adobe Photoshop CC (Adobe Systems, 2014), the faces were digitally cut from the frames and placed against a neutral beige background (RGB model: R=217, G=202, B=126) (Figure 2.1). Faces were cut out from the background and from the rest of the body visible in the frame as we did not want differences in body posture to potentially influence the results. The observer sheep had to use only facial cues to solve the discrimination task. Moreover the ability of sheep to recognise and identify such artificial images had been validated by previous studies (Tate et al., 2006).

#### 2.2.4.1 Ruminating in the home pen

The Photo Sheep were filmed by a familiar experimenter while standing ruminating in their home pen. Prior to the filming session, the animals had been habituated to the presence of an immobile experimenter holding a camera in their home pen across several days. Filming only began once animals stopped paying attention or investigating the experimenter and resumed their activities after less than 2 minutes. Animals had their ears in the frontal plane, showed no flared nostrils or wide eyes and were looking straight at the camera (Figure 2.1, a(i), b(i), c(i), d(i)). This situation was considered to elicit a 'neutral state' in the animals. As defined in Chapter

1 this represents a state of very low arousal (very little movement was observed) and of very low positive or negative valence, that can be observed in relaxed situations.

#### 2.2.4.2 Social isolation

Each photo sheep was isolated in a small pen (4.5 x 4.5 m) with solid walls (approximately 140 cm high) for 90 seconds. No visual contact with conspecifics was allowed, but the pen was located in the same building as the home pen, and so auditory and olfactory contact with other sheep was maintained. Short video clips were recorded by two hidden experimenters, as the presence of familiar humans could have been sought out by the isolated animals. Social isolation is a well established source of stress in sheep (Parrott et al., 1988) and all filmed animals displayed stress-related behaviours such as increased locomotion, high pitched vocalisations, and attempts to escape from the test pen (Vandenhede et al., 1998). This situation was thus considered as inducing an emotional state of negative valence and high arousal (Figure 2.1, a(ii), c(ii)).

#### 2.2.4.3 Aggressive interactions

A trough allowing access to concentrate feed to only one sheep at a time was placed in a test-arena with solid walls (4.5 x 4.5m). Photo Sheep were paired for this situation and all possible pairs were filmed (6 pairs). A given pair of Photo Sheep entered the test-arena simultaneously and were given two minutes to interact while being filmed by two hidden experimenters. In each pair, both Photo Sheep showed agonistic behaviours such as nudges, head threats, head butts, or pushes. Images of faces were created from faces of Photo Sheep filmed frontally and while initiating a bout of aggressive interaction (head threat) (Figure 2.1, b(ii), d(ii)), and this situation was considered to have induced an emotional state of negative valence and high arousal in both sheep.

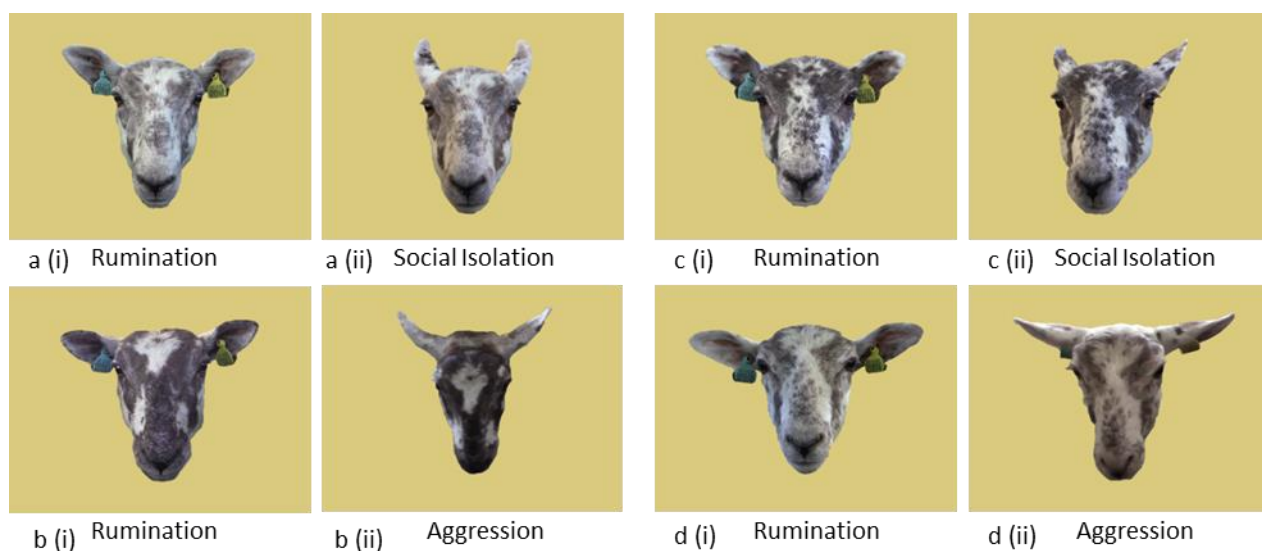


Figure 2.1. Pairs of images obtained from four different Photo Sheep and presented simultaneously in the maze during training and test sessions. (a) and (b) were used during training and (c) and (d) during tests.

### 2.2.5 Discrimination task in a two-armed maze

In the simultaneous discrimination task (Sugnaseelan et al., 2013), sheep had to learn to associate one cue with a positive consequence and a second cue with a negative consequence. Positive reinforcement consisted of a food reward, namely a small amount of concentrate pellets ( $12.5 \pm 1.5$  g) placed in a bucket. Positive punishment consisted of holding the sheep in social isolation for 60 seconds in the incorrect arm of the maze. A bucket containing pellets but closed with a mesh lid was also placed in the incorrect arm, so that the animal could see and smell but not eat the food (Figure 2.2). The type of cue used depended on the phase of training. There was no previous evidence of sheep learning a discrimination task either in a similar set-up or with images of faces. Consequently, if sheep had failed to learn the task with images of faces, it would have been impossible to distinguish between the influences of the new type of cues or of the task itself to explain this failure. Thus, simple coloured cards were used as cues first, to determine whether sheep were capable of learning the discrimination task in the maze. Using cards in the first phase also allowed us to



compare the sheep's behavioural responses when presented with neutral stimuli and with images of faces. The experiment was divided into five consecutive phases: (1) habituation to the maze, (2) training with coloured cards, (3) transition training, (4) training with images of faces, (5) tests with new images of faces (Figure 2.3).

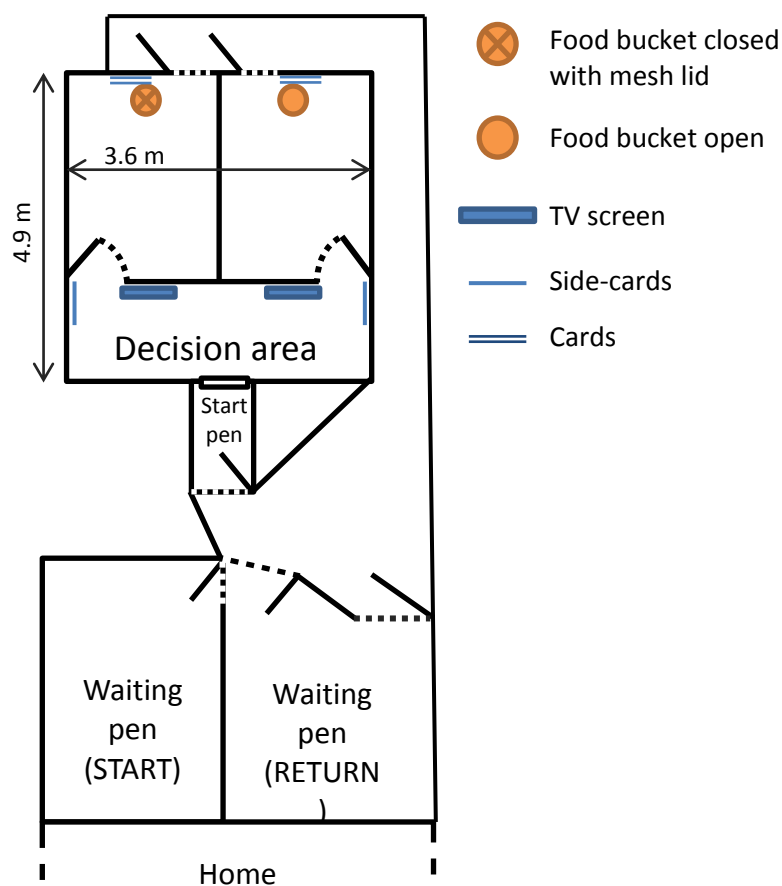


Figure 2.2. Representation of the experimental set up. This includes the two-armed maze, the start pen and the start and return waiting pens, as well as the raceways connecting them. The position of the food buckets alternated between runs depending on which side the rewarded image was placed. 'Side cards' and 'cards' represent the cards where the cues displayed on the screens were repeated on laminated printed A3 sheets (approximately the size of the screen).

## 2.2.6 Experimental set-up

The discrimination task took place in a two-armed test maze (4.8x3.6 m) with solid wooden walls (Figure 2.2). A "start" waiting pen was connected through raceways to a start pen that gave access to the maze was through a start pen a sliding door. A 2.5 m long wooden wall was placed at 1.9 m from the entry gate, with two gates leading to the two arms of the maze. These gates could be closed remotely by an experimenter

standing outside the maze once a sheep had entered one of the arms. This wall also supported two TV screens, one at each end near the gates, and on which two cues were simultaneously displayed. The cue displayed on each TV screen was also shown twice in each corresponding arm: on a card hanging on the wall next to the remotely-closing gate (thereafter referred to as side-card) and on another card placed on the rear wall of each arm. The area between the entry gate and the wall with the TVs was referred to as the decision area, i.e. where the sheep had to choose between the two arms of the maze. Both arms of the maze had an exit door opening onto a raceway leading back to the “return” waiting pen adjacent to the home pen.

### 2.2.7 Habituation to the experimental set-up

The habituation phase was divided into three steps over two days (Figure 2.2). On the first day the sheep visited the maze in groups of three and were allowed to explore it for 3 minutes (all gates remained open). This was repeated three times consecutively per triad in total. The sheep were then split into pairs and entered the maze three times consecutively for 2 minutes. On the second day of the habituation phase, sheep were brought into the maze individually for 1 minute. Again, this was repeated three times consecutively for each individual. One sheep had to be removed from the study at that stage when it became apparent that it was indeed a wether and not a ewe, and thus 35 sheep were included in the next phase of the experiment.

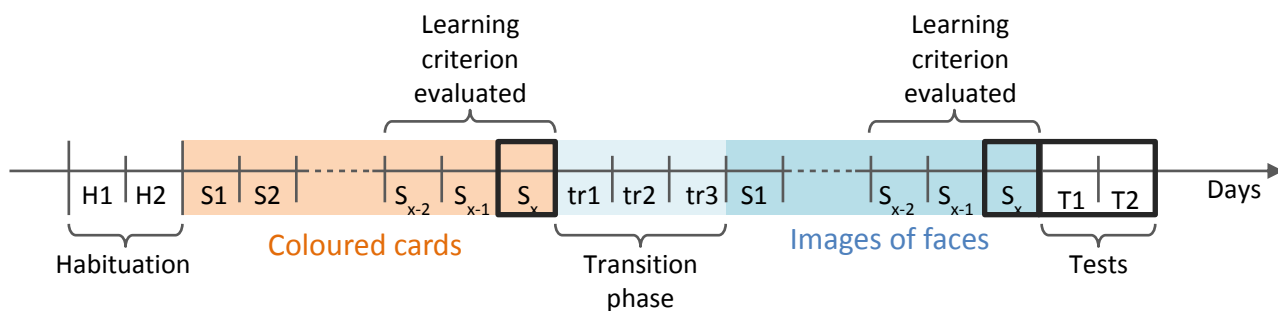


Figure 2.3. Timeline of the five consecutive phases of the study. The total number of sessions needed to reach the learning criterion varied between animals (noted  $x$ ), for Coloured Cards,  $x \leq 18$ , for Images of faces  $x \leq 15$ . The learning criterion ( $\geq 80\%$  correct choices over two

consecutive sessions, or  $\geq 80\%$  correct choices in two out of three consecutive sessions and  $\geq 60\%$  in the penultimate) was evaluated during the three last sessions of both the Coloured Cards and the Image of faces phase, as indicated. Sessions framed by thick black lines were analysed. H = habituation, S = training sessions, tr = transition sessions, T = tests session. During transition sessions, images of faces framed by the corresponding colour (rewarded image was framed by the previously rewarded shade of green and unrewarded image by the previously unrewarded shade) were presented.

## 2.2.8 Training phases

### 2.2.8.1 First training phase: with coloured cards

Two shades of green differing in tone and brightness were used as cues for the first training phase (light: R=240, G=241, B=223; dark: R=122, G=188, B= 50). Sheep can easily distinguish shades of green that differ only in brightness (Doyle et al., 2011). Here we chose cues that also differed slightly in tone to ensure that a good contrast between the two colours would be maintained when displayed on the TV screens.

For half of the test sheep, light green was randomly allocated as the rewarded cue and dark green was the punished cue. The other half of the group received the opposite pairings. The rewarded side alternated to prevent the sheep from place learning. For the first eight runs in the maze, one of the remote-closing gates was closed prior to the sheep's entry, forcing the animal to explore one arm of the maze. This way, sheep experienced the four possible side/reward combinations and their consequences (incorrect-left, correct-right, incorrect-right, and correct-left) twice before making their own choices in the next runs. Side and type alternated, starting with incorrect-left, so that the final run was forced-rewarded. This preliminary conditioning was not taken into account in the analyses.

A training session consisted of ten consecutive runs through the maze. The order of the side/reward combination followed Gellerman (1933) series and was changed after each session to prevent the sheep from learning the order or developing

a side bias. The outcome (side chosen and success or failure) was recorded for each run.

For the first seven training sessions, since all 35 sheep could not be trained on one day, the group was split into two groups of 17 and 18 sheep. Each group went through a training session every other day. After the seventh training session, five animals that had shown consistent side biases were removed from the study and both sets were trained every day. A sheep reached the learning criterion once it had reached a minimum of 80% of correct answers for two consecutive sessions or in two out of three consecutive sessions and above 60% correct answers in the penultimate (i.e.  $\geq 80\%$  -  $\geq 60\%$  -  $\geq 80\%$ ). This learning criterion was based on similar simultaneous discrimination tasks in sheep (Sugnaseelan et al., 2013), and allowed the animals to potentially have a 'bad day' where their performance could be disturbed by external factors, such as noise outside the testing shed. If after 18 training sessions the animals had still not reached the learning criterion, they were excluded from the next phase of the study. 16 sheep were included in the second training phase.

#### 2.2.8.2 Second training phase: with images of faces

In this phase of training, colours were replaced with images of the Photo Sheep faces (Figure 2.1, a, b). A pair of cues consisted of two images of the same Photo Sheep, one taken in the neutral situation and one in one of the two negative situations (SI for social isolation or Aggr for aggressive interactions, see Section 2.4). To identify which type of negative image they were paired with, neutral images from SI-Neutral pairs are referred to as  $N_{SI}$  and neutral images from Aggr-Neutral pairs are referred to as  $N_{Aggr}$ . The type of rewarded image was balanced between the sheep that reached the learning criterion in the first phase ( $n = 16$  sheep). The four types of images (SI, Aggr,  $N_{SI}$ , and  $N_{Aggr}$ ) were allocated so that for half of the test sheep ( $n/2 = 8$  sheep) the correct cue was an image from one of the negative situations (SI,  $n/4 = 4$ ; Aggr,  $n/4 = 4$ ), and for

the other half, the correct cue was an image from the neutral situation ( $N_{Si}$ ,  $n/4 = 4$ ;  $N_{Aggr}$ ,  $n/4 = 4$ ).

Each sheep went through three transition training sessions to facilitate the transfer of the coloured card cues to the facial cues. For the first session (tr1, Figure 2.3), each face was framed by the colour sharing the same attributes, i.e. the now rewarded face was framed by the colour previously rewarded and vice versa. The colour was also repeated on the side-card, and the card placed above the bucket in the arm was a repetition of the framed face. For the next two sessions (tr2 and tr3, Figure 2.3) the coloured side-card was removed but the pictures were still framed in shades of green. No side cards were presented during the training phase with faces, as the orientation of the faces printed on the cards would have been different to the faces presented on the screen. This is why the side cards were progressively removed during the transition training sessions. These three transition training sessions were not included in the number of sessions needed to reach the learning criterion.

From the fourth session onwards the only cues available to the sheep to choose an arm of the maze were images of faces presented on the screens and repeated on the cards above the feed bucket (Figure 2.2). The learning criterion was the same as during the coloured cards phase. As soon as a sheep had reached the learning criterion, it was moved on to the test phase.

### 2.2.9 Test: generalisation to new images of faces

The test phase consisted of two sessions of ten runs each, where the images presented to the test sheep were of the face of another Photo Sheep. The Photo Sheep used in this test phase were also familiar with the test sheep, but images of their faces had never been presented in the maze (Figure 2.1, c, d). The test sheep had to generalise the task they had learned to new images of faces to gain access to the food reward. The type of rewarded image did not change during this phase, e.g. sheep that

had learned to associate SI images with a reward had to associate SI images of a new Photo Sheep with the reward.

### 2.2.10 Data collection and statistical analysis

For both training phases (coloured cards and images of faces), learning speed, i.e. the number of sessions needed to reach the learning criterion, was recorded for each sheep. For every run of the training and test phases, the outcome (success or error), the time from the sheep's entry into the maze (two front feet inside the maze) to its choice (gate arm closed behind the sheep) were recorded (LatChoice, seconds) from video files using The Observer 5.0 (Noldus Information Technology, Netherlands). The observer was blind to the type of rewarded image and rewarded side.

All analyses with non-parametric tests were conducted in Minitab 17 (Minitab Inc., USA). Mixed models were run in GenStat 16<sup>th</sup> edition (VSN International Ltd., United Kingdom). Significance level was set at  $P=0.05$ .

Due to the small number of animals (4 for each type of image rewarded), the two types of negative images (SI and Aggr) and the two types of neutral images ( $N_{SI}$ , when the second image was SI,  $N_{Aggr}$  when the second image of the pair was Aggr) were grouped together under 'negative images' and 'neutral images' respectively for analysis of learning speed. The four types of images were not grouped in other analyses. Data were tested for outliers using Grubb's test at a 5% level of significance and differences in learning speed between the categories of rewarded images were then analysed by Mood median tests, which are more robust than Kruskal-Wallis tests against outliers (Siegel and Castellan, 1988).

Since all sheep needed a different number of training sessions to reach the learning criterion, specific sessions were compared (Figure 2.3, framed in black). The Final Training Sessions (FTS) of both training phases were analysed for all sheep that

reached the learning criterion. The FTS consisted of the last session of a phase, before the animal that had reached the learning criterion moved onto the next phase. The FTS thus did not have the same session number for every animal, but all sheep were at a similar state of training and performance level in the task (correct upon 80% answer). The two test sessions where sheep had to generalise the task were also selected for analyses.

For the selected training and test sessions, the effects of the type of rewarded image (CorrectImage) and the side of the rewarded image (left or right, noted CorrectSide) on the success of a run (0 or 1) were analysed by a general linear mixed model (GLMM) using a binomial distribution and logit link function. CorrectImage and CorrectSide were included as fixed effects in the final model, and Session nested within Animal as random effects.

If sheep chose at chance level during a test session, then the mean value of the success variable was 0.5. In that case, with the logit transformation used by the GLMM the mean would be:  $\text{logit}(0.5) = \ln(0.5/(1-0.5)) = 0$ . To test whether sheep had chosen the correct image at above chance levels during the two test sessions, the confidence interval was calculated for the mean value of the success variable for each CorrectImage, based on the output from the GLMM analysis. If the confidence interval included 0, it was not possible to tell whether the sheep had chosen above chance level.

LatChoice was transformed using a natural log function to conform with statistical assumptions. For the same selected training and test sessions (Figure 2.3), LatChoice was then analysed by REML with repeated measurements, using a power model to account for correlations within subjects across time. CorrectImage, CorrectSide, outcome of the run (Success) and their interactions were included as fixed effects. Random effects included Run and Animal. Post-hoc pairwise comparisons were

done with least significant difference tests. Normality of the residuals was checked graphically.

## 2.3 Results

### 2.3.1 Training with coloured cards

Sixteen sheep (46%) reached the learning criterion in 18 sessions or less. Only two sheep learned the task in less than 10 sessions (7% of total number of sheep or 12.5% of successful sheep) (Figure 2.4). There was no difference in learning speed between sheep that had to associate a light green card with the reward and sheep that had to associate a dark green card with the reward (Medians: 14 vs. 15 sessions,  $\chi^2 = 0.25$ ,  $df = 1$ ,  $P = 0.614$ )

There was no effect of CorrectSide on the number of correct choices during the final training session of the Coloured Cards phase ( $F_{1,157} = 0.26$ ,  $P = 0.613$ ). There was no effect of CorrectSide (left or right,  $F_{1,121.2} = 0.29$ ,  $P = 0.593$ ), CorrectCard (Neutral, SI or Aggr,  $F_{1,37.9} = 0.30$ ,  $P = 0.590$ ), Success (correct vs. incorrect choice,  $F_{1,128.9} = 0.17$ ,  $P = 0.683$ ) or any interaction between those three factors (CorrectSide.Success:  $F_{1,129.3} = 1.23$ ,  $P = 0.269$ ; CorrectSide.CorrectCard:  $F_{1,121.2} = 0.45$ ,  $P = 0.504$ ; Success.CorrectCard:  $F_{1,130.2} = 1.25$ ,  $P = 0.266$ ; CorrectSide.CorrectCard.Success:  $F_{1,131.6} = 0.09$ ,  $P = 0.770$ ) on LatChoice either.



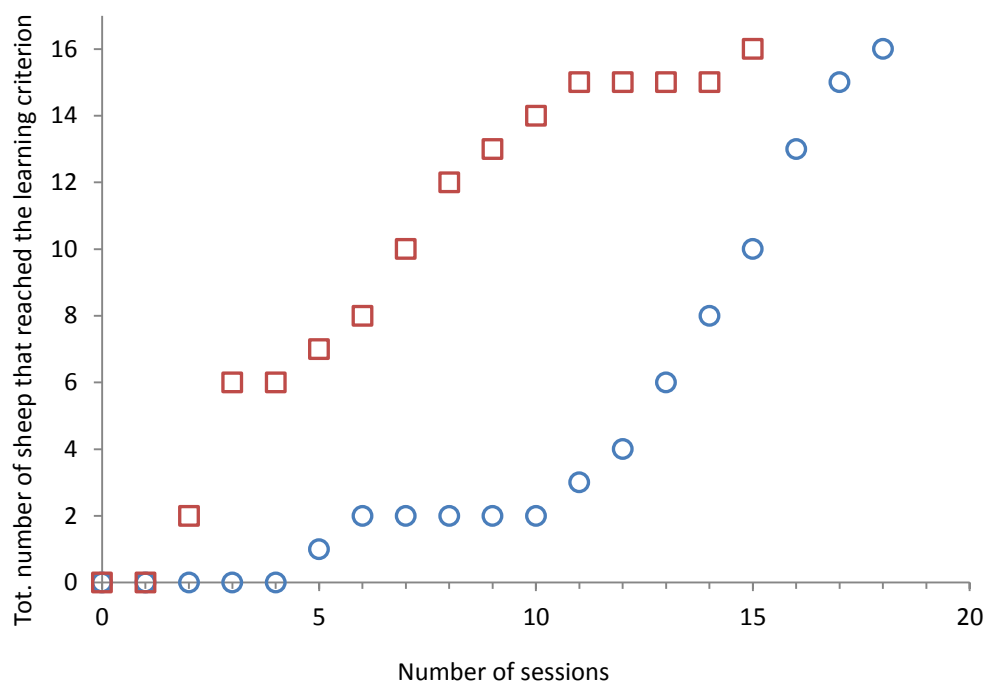


Figure 2.4. Number of sheep ( $n = 16$ ) that had reached the learning criterion for each session. Training phase with coloured cards is coded with blue circles and training phase with images of faces with red squares.

### 2.3.2 Training with images of faces

All 16 sheep (100%) reached the learning criterion with images of faces within 15 training sessions. Fifteen out of 16 animals (94%) reached the learning criterion after eleven sessions (Figure 2.4).

There was a significant difference in learning speed between sheep that had to associate a neutral image ( $N_{Aggr}$  or  $N_{SI}$ ) with the reward and sheep that had to associate a negative image ( $SI$  or  $Aggr$ ) with the reward. Sheep learnt the task faster and needed fewer training sessions when a negative image was rewarded (Medians: 4 vs. 7.5 sessions,  $\chi^2 = 4.00$ ,  $df = 1$ ,  $P = 0.046$ , Figure 2.5). Grubbs' test results showed that there was no outlier ( $G = 1.17$ ,  $P > 0.90$ ).

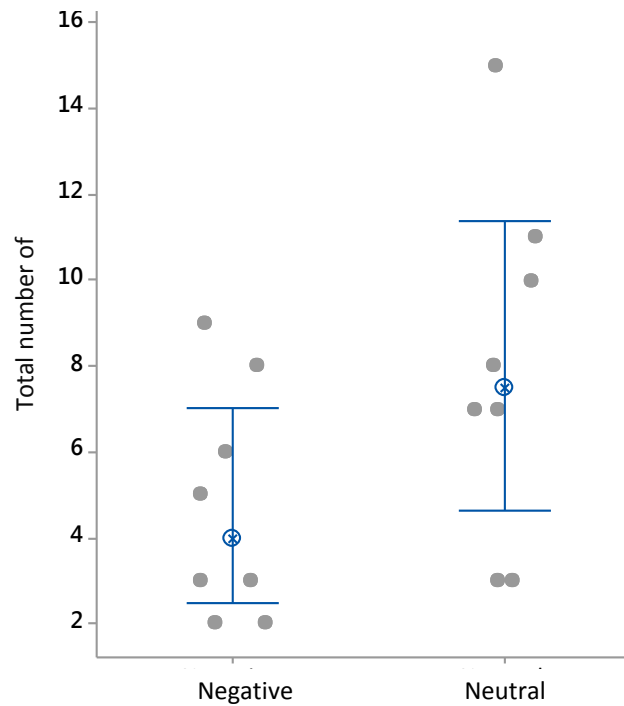


Figure 2.5. Total number of sessions needed to reach the learning criterion by category of CorrectImage (Neutral = NAggr and NSI, Negative = Aggr and SI). Each grey dot represents one individual sheep. Medians of each group are indicated by the blue crossed-circle.

During the final training session, sheep made the correct choice more often when the rewarded image was on the right side of the maze ( $F_{1,152} = 4.10$ ,  $P = 0.045$ , Figure 6a). There was no effect of the type of image rewarded on the sheep's total number of correct choices ( $F_{3,152} = 0.86$ ,  $P = 0.462$ , Figure 2.6b).

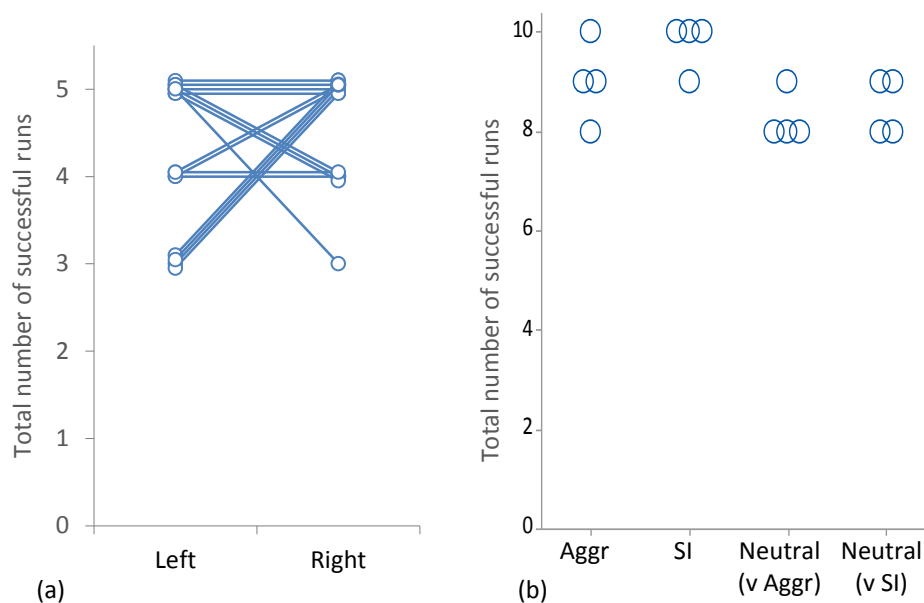


Figure 2.6. (a) Total number of successful runs by CorrectSide for the Final Training Session (FTS) with images of faces. Each line represents one sheep. (b) Total number of successful runs by CorrectImage for the FTS with images of faces. Aggr: aggressive interactions, SI: social isolation, Neutral: ruminating in the home pen.

### 2.3.3 Tests: generalisation to new images of faces

The generalisation of the task to new pairs of images of faces was affected by CorrectImage ( $F_{3,26.6} = 3.37$ ,  $P = 0.033$ ). Based on the confidence intervals, sheep that had Aggr as their type of rewarded image did not choose the correct image at above chance levels (Table 2.1), while sheep that had SI,  $N_{Aggr}$ , or  $N_{SI}$  as their rewarded image did (Table 2.1).

Table 2.1. Confidence interval (CI) of the mean number of successes by CorrectImage. To test whether sheep chose the correct image at above chance levels during the two test sessions, the confidence interval was calculated for the mean value of the success variable for each CorrectImage, based on the output from the GLMM analysis. If the confidence interval included 0, it was not possible to conclude that the sheep chose above chance level. Transformed CI and means are presented, and backtransformed means are included to help with interpretation

	CI Lower bound	CI Higher bound	Mean	Backtransformed mean (mean % of success)
<b>Aggr</b>	-0.671	0.579	-0.046	49%
<b>SI</b>	0.122	1.406	0.764	68%
<b><math>N_{Aggr}</math></b>	0.559	1.937	1.248	78%
<b><math>N_{SI}</math></b>	0.488	1.852	1.170	76%

Across all types of CorrectImage, sheep made the correct choice more often when the rewarded image was on the right ( $F_{1,288.1} = 5.02$ ,  $P = 0.026$ , Figure 2.7). Overall during test sessions, LatChoice was shorter when the correct image was on the right side ( $F_{1,289.3} = 23.92$ ,  $P < 0.001$ ; right:  $8.4 \pm 0.6$  s, left:  $9.6 \pm 0.8$  s). LatChoice was significantly higher when sheep made the correct choice than when they made a mistake (correct choice:  $9.4 \pm 7.8$  s, wrong choice:  $7.9 \pm 10.8$  s;  $F_{1,292.9} = 13.26$ ,  $P < 0.001$ ).

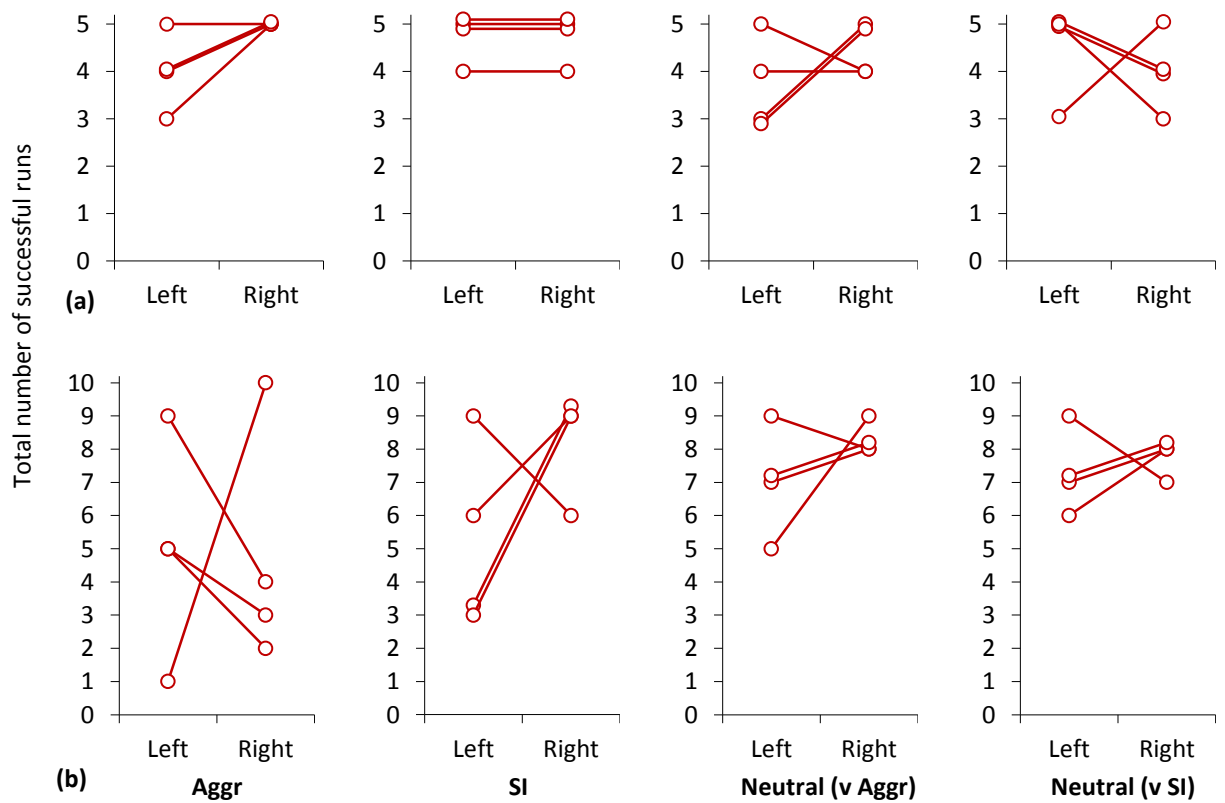


Figure 2.7. Total number of successful runs by Correct Side, i.e. the side where the CorrectImage was presented, and by CorrectImage (a) for the final training session with images of faces (FTS), and (b) for the two test sessions.

## 2.4 Discussion

We investigated whether sheep could discriminate between images of faces taken in situations eliciting emotional states of neutral or negative valence, using a simultaneous discrimination task in a two-armed maze. We also assessed the influence

of the valence of the rewarded image on learning processes. All sheep that learned the discrimination task between coloured cards reached the learning criterion with images of faces. There was no difference in learning speed with coloured cards, however, sheep learnt to associate the food reward with a negative image faster than with neutral images.

### Influence of the type of images of faces on learning processes

As predicted, the type of image rewarded (CorrectImage) had an effect on the learning process, while the type of coloured card rewarded (light or dark green) did not. However, we actually observed the opposite of our hypothesis regarding learning speed. Images of calm conspecifics are approached voluntarily by sheep (Tate et al., 2006), and are thus regarded as primary reinforcers. We had therefore proposed that sheep learning the association between images of ruminating Photo Sheep and food reward would reach the learning criterion faster than sheep learning the association with images taken in negative situations. However, sheep actually learnt the task more quickly when their rewarded image was one of the two negatives images (SI or Aggr).

In humans, negative stimuli (coloured images of beetles, negatively valenced images from the International Affective Picture System (IAPS) database) induce stronger and faster responses (e.g. higher amplitude and shorter latency of electrophysiological markers, shorter response time in key pressing) than positive or neutral stimuli (coloured images of buildings, neutral or positively valence images from the IAPS database) (Mogg et al., 2000; Delplanque et al., 2004). A previous study showed that sheep were indeed more attentive (head turned to the screen for at least 2 s) towards videos showing agonistic interactions between conspecifics than towards videos showing ruminating sheep (Vögeli et al., 2015). In our study, if the attention of sheep was increased towards negative images of faces, this may have aided them to learn to associate an image of a negative face and reward faster. This difference in

learning speed could thus be first evidence that sheep can not only distinguish between facial features (Tate et al., 2006), but that they also perceive the valence of the expression shown on the images. This way, sheep would pay more attention to images of faces taken in negative situations and potentially perceive them as negative. This would represent a first step towards the use of images of faces in cognitive bias studies. Further studies are still needed to determine if the differences in learning performances were due to sheep paying more attention to negative images, or if images taken in situation of high arousal but positive valence would have the same effect.

The generalisation of the discrimination task to new images of faces during tests was also affected by CorrectImage. Only Aggr-rewarded sheep were not able to generalise the task to images of new familiar individuals. Since sheep that had  $N_{Aggr}$  as their rewarded image had no difficulties in generalising the task, the poorer results from the Aggr-rewarded group cannot be explained by an increased difficulty in discriminating the neutral from the aggressive face in the new pair of cues. SI- and Aggr-rewarded sheep reached the learning criterion faster. Consequently, these sheep had been less exposed to images of faces than neutral-rewarded sheep. Having a greater experience of the images stimuli might have helped the sheep in the generalisation of the task to new images. However SI-rewarded sheep were able to generalise the task to new images and did not differ from Neutral-rewarded sheep in their performance in the generalisation. Therefore, the previous experience of images of faces cannot entirely explain the poorer performance from the Aggr-rewarded animals either.

It is also possible that the identity of the Photo Sheep influenced the results from the Aggr-rewarded sheep. If the new Photo Sheep was a very dominant animal, seeing it presenting an aggressive expression might have prompted a strong avoidance response. However, we selected Photo Sheep based on body weights, so that they would be of average body weight. From that perspective, it is quite unlikely that all

sheep from the Aggr-rewarded group were subordinate to the Photo Sheep, but that possibility cannot be excluded. Knowing the hierarchical relationships between the Photo Sheep and the tested animals, would have enabled us to clarify this point and to examine the influence of rank on learning speed.

Lastly, it is worth noting that during test sessions, sheep took longer to make correct choices. The more challenging task of transferring a rule to new cues might explain this variation, since no such difference in latency to choose was observed at the end of the training phase. It also indicates that sheep that made mistakes probably did not take time to process the two cues, but chose a side based on other factors such as a side bias.

### Asymmetries in face-based perception of emotions

In our study, sheep were more likely to make the correct choice and LatChoice was shorter when the rewarded image was on the right side of the maze than when it was on the left. First, it is important to note that faces are not the only type of stimuli inducing a left visual field/right hemisphere bias. For instance, in mammals and birds, novel stimuli, but also negative stimuli, are preferentially processed by the right hemisphere as well, i.e. looked at with the left visual field (reviewed in Rogers, 2010; and Rogers et al., 2013). Visuospatial cues in the environment elicit a left visual field/right hemisphere bias too (Rogers, 2002). This might explain the spontaneous biases observed in different learning situations involving visuospatial cues in ungulates (cattle: Arave et al., 1992; sheep: Erhard et al., 2004; goats: Langbein, 2012). Indeed, for animals with laterally-placed eyes, approaching an object from the right facilitates its observation with the left visual field. Hence, several inherent components of a stimulus can lead to asymmetries in its processing. Since our experimental set-up was not designed to test laterality, the right and left sides of the maze were confounded with other parameters that could lead to side biases (north/south, light gradient, side of the

exit raceway), we cannot conclude that any side bias that we observed is strictly due to an effect of the cue used. However, it is possible to make some interesting observations, while keeping a careful approach in the interpretation of our results.

When using images of faces in our discrimination task, the cues were faces, but also visuospatial, and these are two types of stimuli that have been associated with a left visual field/right hemisphere bias. One of these two characteristics, or a combination of them, could explain the observed right side bias. When turning towards the right arm of the maze, sheep placed both images in their left visual field. This may have facilitated the processing of the pair of cues and made it faster. Since CorrectSide had no effect during the Coloured Cards training phase, the bias observed with images of faces may be attributable to the sheep perceiving them as faces and not to the visuospatial nature of the cues or to environmental factors.

### Methodological limitations

Only 16 out of 35 sheep were included in the second training phase, with images of faces. We allowed sheep a maximum of 180 runs in the maze to learn the task during the Coloured Cards phase. This criterion is within the range of learning performances of sheep in similar tasks that also involved pairs of cues presented alternatively on both sides (80 to 240) (Sugnaseelan et al., 2013). Based on the results from the final training session of the coloured cards phase, given a few more training sessions, more animals would have most likely have reached the learning criterion, but only the faster learners were included in the subsequent phases of the study. Since stress and negative emotional states impede cognitive abilities in sheep (Destrez et al., 2013b; Doyle et al., 2014a), it is also likely that the animals that reach the learning criterion first were less fearful and found repeated handling and isolation less stressful. It is also possible that these animals might have had better cognitive abilities. As such our conclusions may not generalise to the whole population of sheep. Such methods



that involve extensive training phases might also not be the most indicated, as they often leads to the exclusion of a number of animals. However we were still able to establish that sheep can discriminate between images of faces taken in situations of varying valence, and that the valence of the situation influenced the learning speed. It should also be mentioned that due to the small sample size, the effect of the type of coloured card rewarded on learning speed that we identified could have been driven by a single outlying point, however no outliers were identified in the data. Unfortunately, no sample size calculation had been used prior to the start of the experimental work, which would have allowed us to have clearer objectives regarding how many sheep should be brought to the learning criterion. The number of animals used was determined through practical reasons, i.e. how many sheep were available at the experimental farm at the time. Hill breeds of sheep might also not be ideal for cognitive studies due to their high emotional reactivity; lowland breeds such as Clun Forest sheep could be more indicated for such studies. The living conditions specifically associated with hill or lowland breed could also influence their use of visual stimuli, hill breed for instance are usually much more spread out from the other individuals in the flock, and it is possible that they rely more on auditory rather than on visual signals for social recognition (Shillito Walser, 1980).

Finally, Bovet and Vauclair (2000) raised a concern about using “pictorial stimuli” in animal studies without controlling for how images are perceived by the animals. In our study, we were able to confirm that reactions to images of faces differed from reactions to coloured cards in sheep. The differences in learning speed and behavioural lateralisation that we observed only with images of faces suggest that images of faces were perceived as faces by the sheep, but further evidence is needed to draw strong conclusions.

## 2.5 Conclusion

Sheep discriminated images of faces of conspecifics taken either in a negative or in a neutral situation. Sheep were also able to generalise this discrimination to images of new faces, but this ability did not extend to images taken during aggressive interactions: sheep from the Aggression-rewarded group were unable to distinguish between images of faces above chance. Sheep reacted differently depending on the valence of the emotional state displayed by the Photo Sheep and learning was affected by the type of emotion displayed. This suggests that sheep are able to perceive the valence of the emotional state displayed on an image of a face, which is an encouraging first step for the use of images of faces in cognitive bias studies.

## Chapter 3: Housing conditions affect the spontaneous reactions of sheep to videos of conspecifics in situations of varying valence

## Abstract

The interaction between mood and emotions in humans and animals has been a subject of growing attention. Emotional states can be perceived by other individuals, whose behaviour can change in reaction to this perception. We investigated sheep spontaneous reactions to videos of familiar conspecifics in situations of varying valence (negative: social isolation and aggressive interactions, neutral: ruminating in the home pen). Prior to the test, half of the animals were exposed to 4 weeks of unpredictable negative housing conditions while the control group lived under standard housing conditions. We expected that the negative housing conditions would induce negative mood state. Sheep were then presented with 30 s long video clips, and their ear postures and interactions with the screen were recorded. The impact of negative housing conditions was assessed by faecal glucocorticoids concentration and a novel object test. Results were analysed using REML. Sheep touched the screen more when presented with videos of social isolation compared to aggressive interactions ( $F_{2,101.9} = 4.26$ ,  $P = 0.039$ ) or rumination ( $F_{2,101.9} = 4.26$ ,  $P = 0.006$ ). Housing conditions had no detectable effect on faecal glucocorticoid concentrations ( $F_{1,34} = 0.36$ ,  $P = 0.555$ ) or on the behaviours during the novel object test. Housing conditions, however, had an effect on spontaneous reactions of sheep to the videos: sheep from the negative housing conditions displayed more ears forward ( $F_{2,109.3} = 3.06$ ,  $P = 0.049$ ) and tended to show less ears backward ( $F_{2,113.5} = 2.84$ ,  $P = 0.063$ ) when looking at negative videos, and especially videos of aggressive interactions. Sheep reacted differently to videos of familiar conspecifics in situation of varying valence; it can thus be concluded that they perceived the situations differently. Our results suggests that negative housing conditions influenced the responses of sheep to emotional stimuli.

### 3.1 Introduction

Emotions can be defined as short-term affective states (Parkinson, 1995), and are differentiated from long-term affective states, or mood (Plutchik, 1994; Mendl et al., 2010). The influence of both short-term emotional states and mood on the perception of external stimuli has been demonstrated in a wide range of species (Paul et al., 2005). This has led to the development of the cognitive bias methodology which uses the effect of emotions or mood on cognitive processes (judgment, attention, or decision making) to evaluate the emotional state of the subject (Mendl et al., 2009). For instance, and very generally, individuals in a negative emotional state are more likely to make 'pessimistic' choices, and individuals in a positive emotional state are more likely to make 'optimistic' choices (for a review in animals see Roelofs et al., 2016a). Long-term affective states or mood can also affect short-term emotional responses, and the interplay between mood and emotions has been the subject of several scientific studies. In humans, low-mood states have been shown to alter perception of emotions in photographs of familiar scenes (Isen and Shalcker, 1982) and in music (Vuoskoski and Eerola, 2011). Similarly, sheep that had been exposed to housing conditions assumed to induce a negative mood reacted more negatively towards negative situations (social isolation) and more positively towards positive situations (pleasurable grooming) (Reefmann et al., 2012).

The impact of emotional states on animal welfare has been widely assessed for individual animals (Désiré et al., 2002; Boissy et al., 2007; Fraser, 2009) but the consequences for the social group as a whole are still far from fully understood. Emotions can indeed be perceived by other individuals, and their behaviour can change in reaction to this perception of emotions, either positive or negative (Spinka, 2012). This social dimension of emotions has been widely studied in humans. For instance, in depressed or anxious patients, perception of emotional social signals such as facial expressions is altered and neutral faces are perceived as sad or threatening (Leppänen

et al., 2004; Demenescu et al., 2010). From an animal welfare point of view, if animals in a negative emotional state have an altered perception of social emotional stimuli, it may compromise their ability to correctly interpret the behaviour of conspecifics, potentially leading to difficulties to avoid aggression or painful situations, to establish social bonds, or to integrate into a social group,. On the other hand, animals in a positive mood might be able to cope better with short-term negative events (Reefmann et al., 2012) and provide social support (buffering) to flock members (Rault, 2012).

Manipulation of housing conditions has been used to induce mood changes in rodents and livestock species. Stimulus-poor housing conditions, combined with unpredictable negative events have been shown to induce chronic-stress states similar to human depression in sheep (Destrez et al., 2013b) and rodents (Harding et al., 2004). Conversely, enrichment of the environment and good human-animal relationship have been shown to induce states similar to positive mood in animals (starlings: Matheson et al., 2008; sheep: Reefmann et al., 2012; pigs: Douglas et al., 2012).

We investigated spontaneous reactions to videos of familiar conspecifics in situations of varying valence in sheep (negative: social isolation and aggressive interactions, neutral: ruminating in the home pen). An attention bias for negative stimuli has been identified in mammals (Carretié et al., 2001), and we thus predicted that if sheep perceived videos of conspecific videos of conspecifics experiencing negative situations as negative stimuli, they would be more attentive towards these videos compared to videos of ruminating conspecifics. Half of the animals were exposed to 4 weeks of unpredictable negative housing conditions (adapted from Destrez et al., 2013a), while the control group lived under good standard housing conditions. We expected that negative housing conditions would induce a chronic-stress state resulting in a low-mood state in the sheep, and that standard housing conditions would not have an effect on the mood state of the animals. We used

physiological (faecal glucocorticoids concentrations) and behavioural indicators to assess the impact of the affect-manipulation treatment on the sheep emotional state. We then hypothesised that negative housing conditions would thus have an effect on the reactions of the sheep to emotional stimuli, and that animals from the negative housing condition group to react more strongly to videos of negative situations (Reefmann et al., 2012).

## 3.2 Material and methods

### 3.2.1 Animal ethics

This study was approved by the SRUC Edinburgh Animal Ethics Committee (ED-AE-2-2014). The animals included in the negative housing conditions group were placed under Home Office License regulation for moderate severity (Welfare of ruminants, project number: 60/4081). All animals were weighed weekly before, during and after the study, and were subjected to regular veterinary examinations as part of the farm schedule. No animals had to be removed from the study due to illness or injury.

### 3.2.2 Animals and housing

This study took place at the SRUC Woodhouselee experimental farm in Edinburgh (UK) in October 2014. Thirty-nine Scottish Mules ewes aged 16 to 18 months ( $54.8 \text{ kg} \pm 4.2 \text{ kg}$ ) were used in this experiment. These animals had been born and raised on the SRUC experimental farm and were familiar with each other, having lived together for at least six months prior to the trial. The sheep had participated in the experiment presented in Chapter 2 and were therefore accustomed to human handling at this stage. Three ewes were pseudo-randomly selected based on body weight so that their body weight was similar to the average body weight of the group ( $50 \pm 2.1 \text{ kg}$ ) and are thereafter referred to as Video Sheep (see Section 2.5). The Video

Sheep were not tested but were housed with the rest of the group for the duration of the trial. Video Sheep were split between housing conditions groups, and switch group every week so as to ensure similar levels of contact and familiarity in both groups.

Sheep were housed together, indoors in a straw pen (10 x 5 m). They were fed *ad libitum* hay and supplemented with concentrate pellets (0.5 kg per sheep per day). They had unrestricted access to water.

### 3.2.3 Housing conditions treatments

The 36 tested sheep were randomly assigned to a negative housing conditions group (n=17) and a control group (n=19). The negative housing conditions group was exposed to unpredictable and uncontrollable negative events during four weeks. In the meantime the control group was housed under good standard farming conditions and in a different shed. Pen space allowance for the control group was 3.5 times higher than DEFRA minimum recommendation (2.8 m<sup>2</sup> per sheep), and so was feed space allowance (60 cm).

The protocol used with the negative housing conditions group was adapted from Destrez et al. (, 2013a) to fit with the facilities available at the farm (e.g., no foot bath available) and was reduced to four weeks, as this had been shown to be a sufficiently long exposure by previous studies (Doyle et al., 2011). Negative events were split into three categories: predator cues, conspecific cues and human cues (Table 3.1). Negative events that did not require handling could take place either during the day or during the night (indicated by a \* in Table 3.1). During each aversive human interaction, the experimenters wore yellow high-visibility jackets. During regular farming procedures and tests they were dressed in blue overalls. The experimenters were the same throughout the four weeks, except for the weekly exposure to a noisy unknown human, when a different unfamiliar person was recruited every week.



Table 3.1. Description, frequency and duration of aversive events used in the Negative housing conditions group. Predator cues were not delivered in a set and at the same time, but in a random order. \* indicates events that could take place either during the day or during the night.

Event	Frequency	Duration
<b>Predator cues</b>		
Dog bark and wolf howls during the night*	Once a week	4 sessions of 15 min
Aversive contact with an unknown dog*	Once a week	30 min
Odours of dog faeces*	Once a week	24 h
<b>Conspecific cues</b>		
Restricted feed space (25 cm per ewe, ~80% of recommended DEFRA allowance)	Constant	-
Restricted pen space (0.8 m <sup>2</sup> per ewe, minimum pen space recommended by DEFRA for non-pregnant hill ewes)	Constant	-
<b>Human cues</b>		
Individual restraint (sheep were weighed and BCS scores taken before this procedure)	Once every 10 days	5 min per sheep
Group restraint in a pen similar to those used during routine handling (4x2.5 m)	Once a week	1h
Presence of a noisy unknown human (shouting and waving arms to move the animals but no contact)	Once a week	15 min
Unpredictable/delayed feeding times	Twice a week	-
Wet bedding for a limited period of time	Once a week	4h
Lights on during the night*	Once a week	4 sessions of 30 min
Fan blowing*	Once a week	5 sessions of 15 min over 24 hours
Restraint in a stock truck	Every 3 weeks	30 min

### 3.2.4 Faecal glucocorticoids metabolites

Faecal glucocorticoids metabolites (FGM) were measured to determine whether the negative housing conditions, that involved exposure to stressors, induced changes in the HPA-axis activation compared to the control condition. Faecal samples were collected after defecation on the final day of the affect manipulation treatment in both groups. When samples could not be obtained on that day, samples were collected during the first day of the test phase. One sheep from the negative housing group could not be collected. Samples were frozen immediately after collection and stored in zip-lock plastic bags at -20°C until analysis. FGM levels were extracted and analysed by enzyme immunoassay (EIA) (for more details, see Palme et al., 2013). For the

extraction, 0.5 g of faeces was transferred to a 15 ml tube and 5 ml of 80% methanol was added. The tubes were then vortexed (agitated) for 30 min and then centrifuged at 2500 g for 15 min. The supernatant was then diluted at 1:10 in assay buffer. The FGM solutions was distributed on EIA plates and read on a spectrophotometer (Thermo Scientific Multiskan FC MicroplatePhotometer) at 450 nm. Quality control samples were included on all EIA plates for intra- (CV = 7%) and inter-assay (CV = 19%) variation coefficients. FGM concentrations were then standardised to the weight of faeces used for extraction (ng per g of faeces).

### 3.2.5 Video stimuli

The video stimuli for this study were created using the clips filmed during our first experiment, from which images of faces of sheep had been extracted. The three situations are briefly presented below (see Chapter 2 for more details). A video clip corresponded to videos of one individual only. There were two Video Sheep: Video Sheep 1 and 2. The two other filmed sheep were not included in this study since the tested sheep were only exposed to each type of video twice. Video Sheep 3 was only paired with Video Sheep 1 and 2 for the aggressive interactions videos. Videos clips were presented to the tested animals without sound, so their selection was based only on visible behaviours and vocalisations could not be heard in the test pen.

After review of the video clips collected, the most representative behaviours observed during the situation were identified (e.g., increased locomotion for the social isolation). This consisted of behaviours consistently presented by all individuals filmed during the situation. The behaviours also had to occur during the same time-window for all individuals (e.g., within 50 sec of the beginning of the isolation). The duration of the video clips (30 s) was based on the duration of presentation in the test pen.

### 3.2.5.1 Ruminating in the home pen

Each Video Sheep was filmed in the home pen by a familiar handler equipped with a HD camcorder (Legria HFM52, Canon, Tokyo, Japan). The Video Sheep was standing and ruminating in a neutral posture with ears in the frontal plane, eyes and nostrils not dilated, and looking straight at the camera. Other sheep were visible on the videos, but not facing the camera (Figure 3.1a). The videos were then edited to produce a 30 s clip for both Video Sheep and are thereafter referred to as 'Neutral videos'.

### 3.2.5.2 Aggressive interactions

A food trough allowing only one sheep at a time to feed, and containing a small amount of concentrate feed (15g), was placed in a test-arena with solid walls (4.5 x 4.5m). A pair of Video Sheep entered the test-arena simultaneously and the pair was filmed for 2 minutes by two hidden handlers. Video Sheep 1 and 2 were both paired with the same individual, Video Sheep 3. Video Sheep 3 was only filmed during the aggressive interactions videos. The most aggressive bouts (head butts, displacements, threats), were then extracted to produce short video clips (30 s), hereafter referred to as 'Aggr videos'. The two sheep were visible on the screen (Figure 3.1b).

### 3.2.5.3 Isolation Stress

The Video Sheep was isolated in a pen with solid walls for 90 seconds. Sequences selected for the 30 s video clip (SI videos) corresponded to the period of highest activity (increased locomotion, escape attempts, defecation/urination). Front and side views of the Video Sheep were visible on the video clips (Figure 3.1c).



Figure 3.1. Screenshots taken from the video clips presented to the sheep in the test pen. (a) Neutral: sheep filmed while standing and ruminating in the home pen. (Video Sheep 1 (a,i) and Video sheep 2 (a,ii)). (b) Aggressive interactions: sheep filmed while competing for access to food. Video Sheep 1 (b, i) and Video Sheep 2 (b, ii) were paired with the same individual for these interactions. (c) Social isolation: sheep were filmed while isolated in a test pen with solid walls for 90 sec (Video Sheep 1 (c, i) and Video Sheep 2 (c, ii)).

### 3.2.6 Tests

#### 3.2.6.1 Experimental set-up

The test pen was located in an independent shed, approximately 30m away from the two housing sheds. All sheep from one housing conditions group were brought to the testing shed every other day by freely following an experimenter shaking a bucket of concentrate pellets. Within the shed, they were placed into a waiting pen (5 x 2m) where they had access to water and hay. From the waiting pen, the animals could be brought individually to a buffer pen adjacent to the test pen (Figure 3.1).

The test pen consisted of a rectangular enclosure (2.4 x 0.8 m) with solid wooden walls and concrete flooring. To display the video clips, a TV screen (19") was placed at eye-level for sheep on the wall opposite the entrance (Figure 3.2).

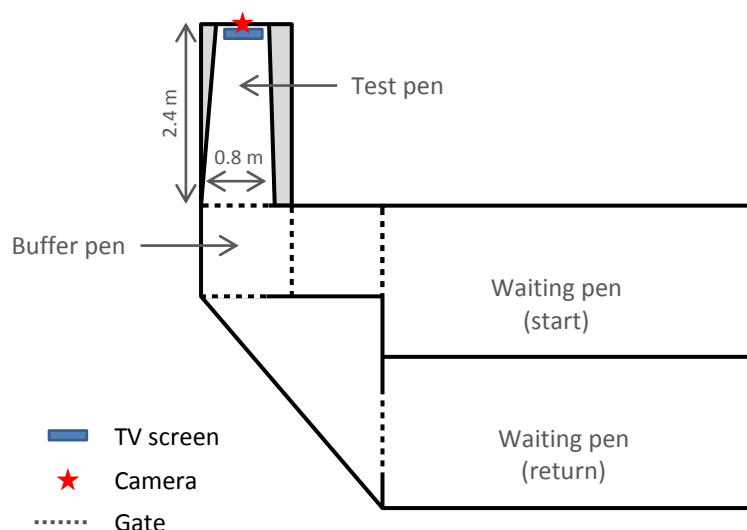


Figure 3.2. Experimental set-up

### 3.2.6.2 Habituation

The habituation period was divided into three phases and took place over the last ten days of the affect manipulation phase for both groups. Firstly one group at a time was walked to the test shed and stayed in the waiting pen for 30 minutes once. For the second phase, sheep from one group were walked to the test shed and placed in the waiting pen, then each sheep was brought individually into the buffer pen and then into the test pen for 30s once, before returning to their home pens.. For the third phase of habituation, sheep were brought to the test shed again and went into the test pen twice, with the second visit taking place once all other sheep had been through it once, resulting in approximately 1 hour between two visits of the test pen. During this habituation phase, the TV screen was displaying a video of an empty pen.

### 3.2.6.3 Spontaneous reactions to videos of conspecifics

Tests took place across four consecutive days, after the fourth week of the affect manipulation phase (Figure 3.3). Due to time constraints, and to allow the animals enough time to rest and feed daily in their home pens, only half of the sheep from each affect manipulation groups were tested on a given day test day (Figure 3.3). Repartition between the two half groups was balanced based on weight. The two first half groups (Control 1 and Negative Housing 1) were presented with videos first, then the second half groups were tested the next day (Control 2 and Negative Housing 2). Control and Negative Housing groups were tested alternatively on consecutive test days, so that the same group was not always tested first.

Sheep were exposed to three videos per day (one of each type) on two different days (Figure 3.3). Aggr, SI and Neutral videos were presented for Video Sheep 1 and 2 to every test sheep. Order of sheep exposure to the videos and order of presentation of the videos for each sheep were balanced for identity of Video Sheep, type of videos (TypeVid) and type of previous video seen (PrevVid) as far as possible.

Video clips had been selected to present homogeneous behaviours across the 30 s and were displayed on a loop on the screen. This way, the video was visible from the moment the animal entered the test pen. For each video presentation, a sheep was brought to the buffer pen and could then enter the test pen (Figure 3.2). The timer started once the sheep had its four legs inside the test pen. At the end of the 30 seconds of video presentation, the animal was released into the return waiting pen, and another sheep was brought into the buffer pen. Fifteen minutes after the last sheep had seen the first video of the day the first sheep was tested again to start the second session of video presentations, resulting in an interval of approximately 1 hour between two video presentations for each sheep.

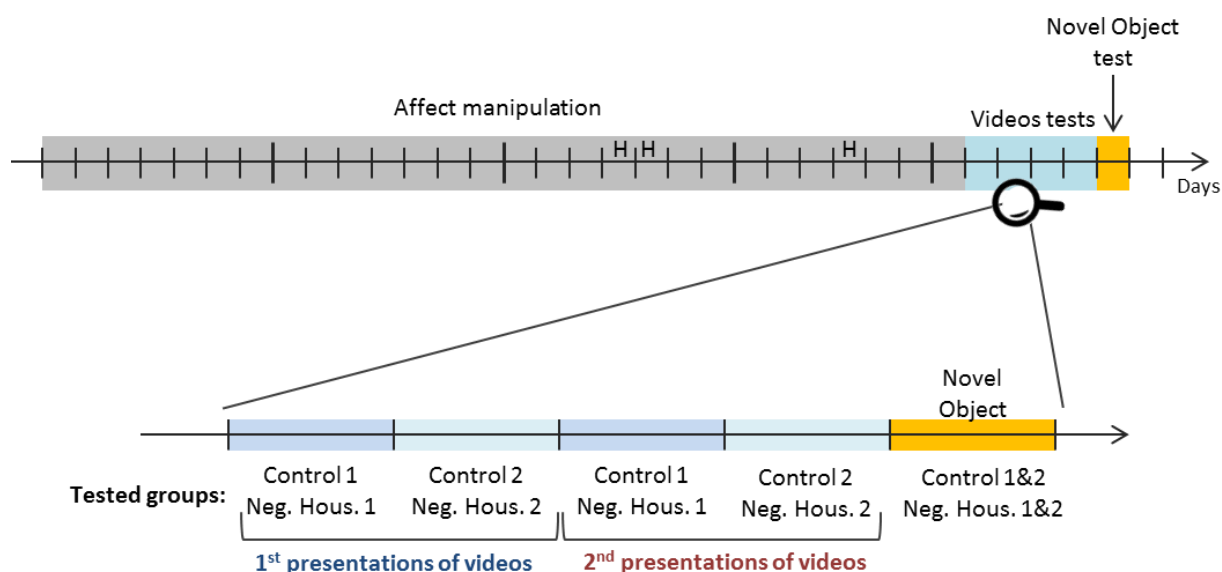


Figure 3.3. Timeline of the experiment. Neg. Hous.: group (n=17) exposed to negative housing conditions during 4 weeks. Split into two subgroups for video presentations (Neg. Hous. 1 (n=9) & 2 (n=8)) Control: Group (n=19) exposed to standard housing conditions during 4 weeks. Split into two subgroups for video presentations (Control 1 (n=10) & 2 (n=9)). 'H' indicates the days where the habituation phases took place.

#### 3.2.6.4 Novel object test

Sheep were subjected to a novel object test to assess possible differences in emotional reactivity between the negative housing conditions and control group. This Novel Object test took place after the last day of video presentations (Figure 3.3). An orange traffic cone was placed in front of the screen in the test pen that was now a familiar environment. The same video of an empty pen used during habituation was displayed on a loop on the TV screen during this test.

Sheep were brought to the test pen as during video presentation sessions, and were given one minute to explore the novel object. Time spent looking at the exit door, the cone or other areas of the test pen as well as time spent sniffing/touching the cone were recorded.

Table 3.2. Recorded behaviours and transformation applied to outcome variables in sheep when shown videos of familiar conspecifics and during a novel object test.

Behaviour		Description	Transformation
<b>Video presentation tests</b>			
<b>Start test</b>		As soon as the sheep was facing the screen	---
Ear postures	Forward	Tip of both ears pointing towards the front of the sheep	Logit
	Backward	Tip of both ears pointing towards the back of the sheep	Logit
	Asymmetrical	Right and left ears in different position regarding a perpendicular to the head-rump axis	Logit
	Horizontal	Tip of both ears in a central posture, along a perpendicular to the head-rump axis	Logit
Looking at screen		Two eyes and head in direction of the screen, regardless of the direction of the body. This is a conservative description of 'looking' considering the wide visual field of sheep (~320°)	Logit
Touching screen		Nose or lips touching the screen	Logit
Approach screen		Walking towards the screen while staring at it or if the sheep was already standing close to the screen, forward movement of the neck while staring at the screen that brought the head closer to the screen.	Binomial (0 = didn't approach, 1 = approached)
Withdraw from screen		Walking away from the screen (backwards) while staring at it, or if the sheep was already standing close to the screen, backward movement of the neck while staring at the screen that brought the head further away to the screen.	Not observed
<b>Novel Object test</b>			
Looking at	novel object	Two eyes and head in direction of the novel object, regardless of the direction of the body	Logit
	exit door	Two eyes and head in direction of the exit door, regardless of the direction of the body	Logit
	other	Two eyes and head in direction of other elements of the test environment, regardless of the direction of the body	Logit
Touching novel object		Nose or lips touching the novel object	Logit



### 3.2.7 Data collection and statistical analysis

Behaviours described in Table 3.2 were scored from the video recordings for video presentation tests and for the novel object test using The Observer 5.0 (Noldus Information Technology, Netherlands). The observer was blind to the type of video shown and time point. Effectively, sheep could see the screen and react to the videos as soon as they were facing it, even if the door was not closed behind them yet. This was taken into account in the video analyses, and behaviours described in Table 3.2 were recorded as soon as the animal was facing the screen. The test ended when the door of the test pen was opened again. The duration of the test thus varied slightly between animals (average duration:  $40.7 \pm 5.7$  s). Percentages of time spent interacting with the screen were calculated for this duration. Both ears were not always visible, for example when a sheep lowered its head while facing away from the camera, or turned its head to one side. Percentages of time spent in a given posture were thus calculated by taking into account the duration when the ears were visible only (on average, at least one ear was out of view for  $11.4 \pm 12.7\%$  of the total duration of the test). Horizontal ear postures did not occur, so this behaviour was not included in the analyses. Due to a failure in recording systems, data from the first day of video presentation was irretrievable, and thus not included in further analysis. 'Withdraw from the screen' was not observed and was thus not included in further analyses.

All analyses were run in GenStat 16<sup>th</sup> edition (VSN International Ltd., United Kingdom). Significance level was set at  $P=0.05$ . All results are presented as boxplots of the untransformed data, unless otherwise stated.

Outcome variables from video presentation tests measured in percentages were logit-transformed to conform to normality and homogeneity assumptions. They were analysed by linear mixed models (REML) with repeated measurements. A power model was used to account for correlations within subjects across time. Heterogeneity

of the variance was allowed when it led to a smaller deviance of the model (one-tailed test with a  $\chi^2$  distribution). The number of approaches of the screen was transformed to binomial variables (0 = did not occur, 1 = occurred) and analysed by GLMM with a binomial distribution, and logit as link function. Residuals were checked graphically for normality.

Housing conditions group (Housing), type of video shown (TypeVid), identity of the Video Sheep (IDVS) and type of previous video shown (PrevVid) were included as fixed effects in the original model for each outcome variable, as well as the interactions between TypeVid, Housing and IDVS. Fixed effects were then fitted by backward stepwise selection, and so not all of the fixed effects above were included in the final model for each outcome variable (see Table 3 for a description of the final fixed effects considered simultaneously). When a predictor was not included in the final fitted model, no statistical results were presented for this predictor. Animal and Session were included in the random effects as Animal nested within Session for the REML and Animal and Session for the GLMM. Post-hoc analyses were conducted using Fisher's least significant difference tests.

For the novel object test, outcome variables (Table 3.2) were logit-transformed and analysed with REML. Housing was included as a fixed effect and Animal as random effect.

Raw data for faecal glucocorticoids concentrations (ng/g of faeces) conformed to homogeneity of variance and normality assumptions and were thus analysed with a one-way ANOVA with Housing as factor and Animal as block.

Table 3.3. Fixed effects included in the final fitted model for each outcome variable for video presentation tests

Outcome variable	Type variable	Fixed effects final fitted model <sup>1</sup>
<b>Ears forward</b>	Percentage	Housing + TypeVid + Housing .TypeVid + PrevVid
<b>Ears backward</b>	Percentage	Housing + TypeVid + Housing .TypeVid
<b>Ears asymmetrical</b>	Percentage	TypeVid + IDVS + TypeVid.IDVS + PrevVid
<b>Staring at screen</b>	Percentage	TypeVid + IDVS + PrevVid
<b>Touching screen</b>	Percentage	Housing + TypeVid
<b>Approached screen</b>	Binomial	TypeVid + PrevVid

<sup>1</sup> Housing: housing conditions group (negative or control); TypeVid: type of video shown, ruminating in the home pen, social isolation or aggressive interactions; PrevVid: type of video shown in the previous test session; IDVS: identity of the Video Sheep

### 3.3 Results

#### 3.3.1 Spontaneous reactions to videos

There was no effect of TypeVid on the different types of ear postures (Forward:  $F_{2,108.3} = 0.30$ ,  $P = 0.739$ ; Backward:  $F_{2,112.6} = 0.33$ ,  $P = 0.722$ ; Asymmetrical:  $F_{2,100} = 0.33$ ,  $P = 0.722$ , Table 3.4). However, the interaction between TypeVid and Housing had an effect on time spent with the ears forward (Figure 3.4a). Sheep from the negative housing group spent less time displaying forward ear postures than sheep from the control group when exposed to Aggr videos ( $F_{2,109.3} = 3.06$ ,  $P = 0.049$ , Figure 3.4a). That same interaction also had a marginally significant effect on time spent with the ears backward ( $F_{2,113.5} = 2.84$ ,  $P = 0.063$ , Figure 3.4b): when the responses of sheep from the negative housing group were considered alone, sheep spent marginally more time with the ears backwards when exposed to an Aggr video than to SI videos ( $F_{2,113.5} = 2.84$ ,  $P = 0.057$ ) or Neutral videos ( $F_{2,113.5} = 2.84$ ,  $P = 0.086$ ).

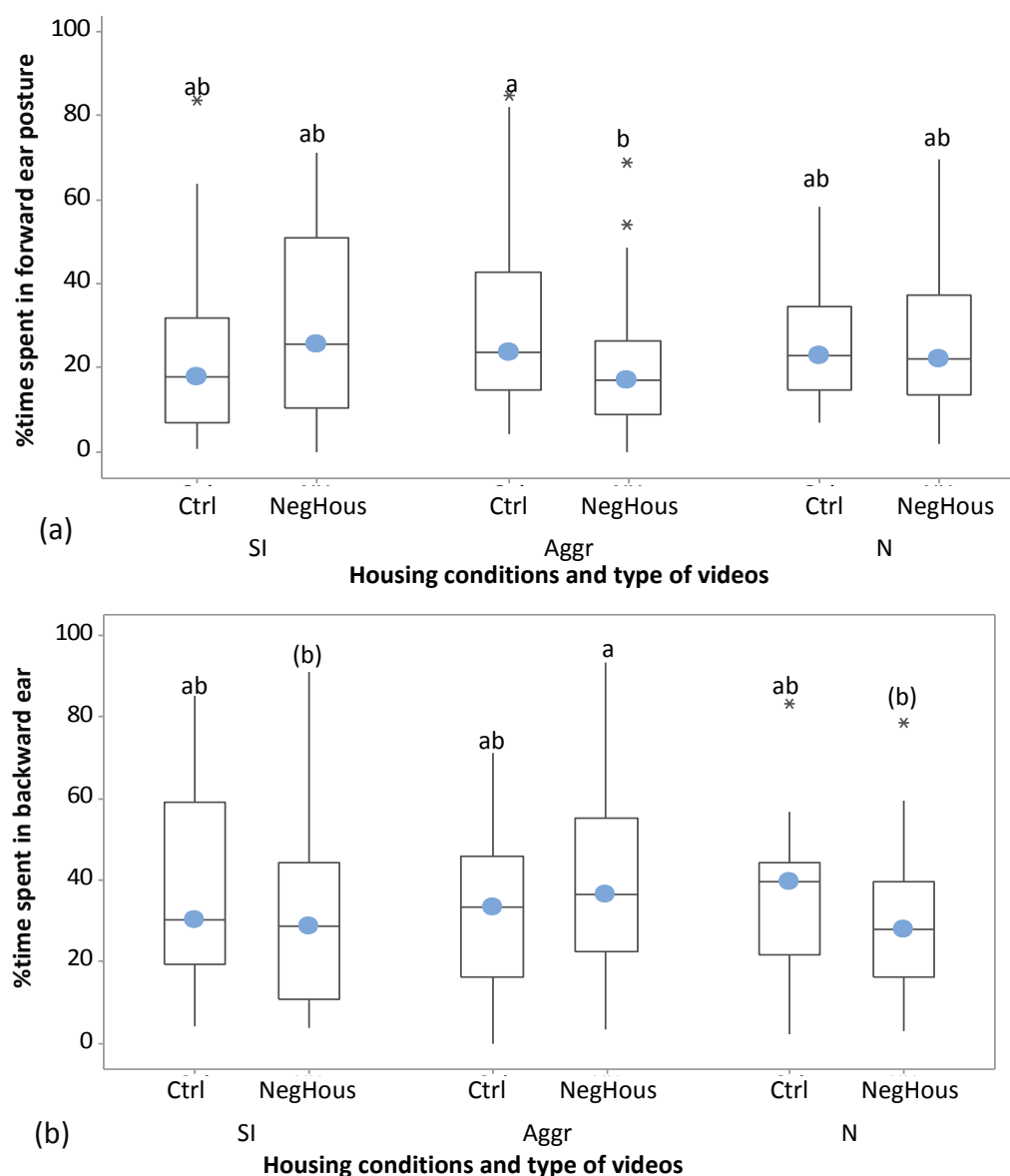


Figure 3.4. **Effect of TypeVid and Housing conditions on (a) time spent with the ears forward** (Ctrl: control group, NegHous: sheep exposed to negative unpredictable events for 4 weeks) **and (b) time spent with the ears backward.**

SI: social isolation, Aggr: aggressive interactions, N: neutral, Ctrl: control group, NegHous: sheep exposed to negative unpredictable events for 4 weeks.  $P < 0.05$  when two bars share no common letters,  $P < 0.10$  when two bars share no common letters, but in brackets. For each group, the median is indicated by the blue dot, the box represents the first and third quartiles, the upper whisker extends to the highest data value within the upper limit and the lower whisker extends to the lowest value within the lower limit. Values noted by an asterisk beyond the whiskers are outliers.

The interaction between TypeVid and the identity of the Video Sheep had an effect on the time spent with the ears asymmetrical. When presented with SI videos, sheep spent less time with their ears asymmetrical when exposed to videos of Video Sheep 1 compared when exposed to videos of Video Sheep 2 ( $F_{2,115.5} = 2.89$ ,  $P = 0.002$ ,

Figure 3.5). Time spent in asymmetrical ear postures did not differ across different Video Sheep for Aggr videos ( $F_{2,115.5} = 2.89$ ,  $P = 0.685$ ) or Neutral videos ( $F_{2,115.5} = 2.89$ ,  $P = 0.227$ ) (Figure 3.5). When presented with videos from Video Sheep1, test sheep also spent marginally less time with asymmetrical ears when presented with SI videos compared to Aggr videos ( $F_{2,115.5} = 2.89$ ,  $P = 0.069$ ) but not Neutral videos ( $F_{2,115.5} = 2.89$ ,  $P = 0.162$ ) (Figure 3.5).

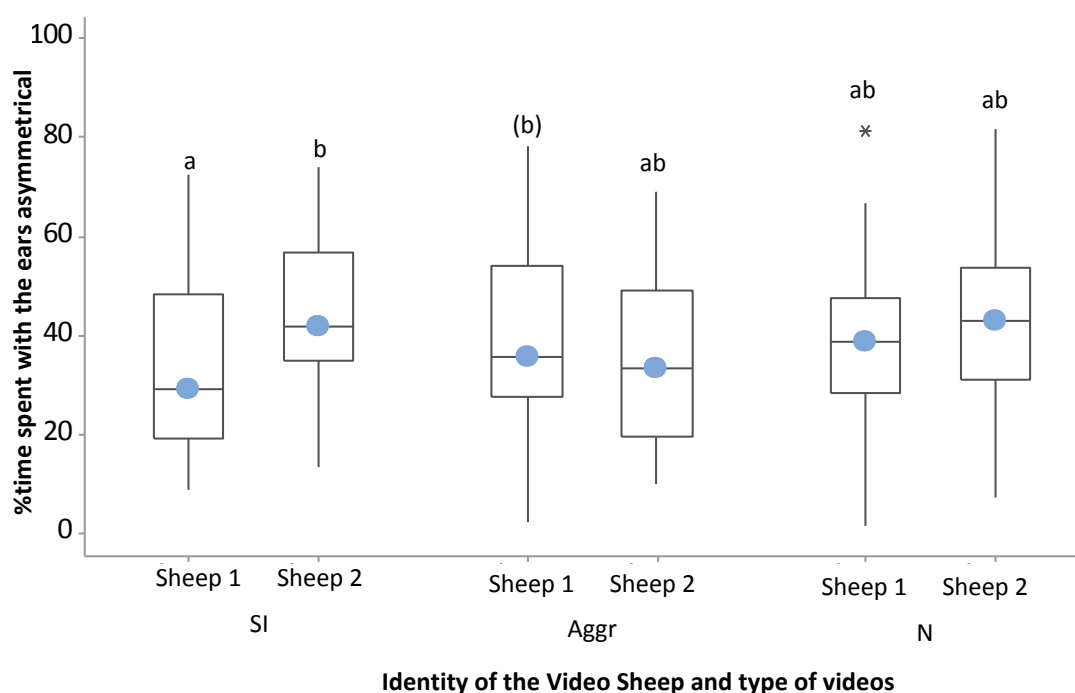


Figure 3.5. Effect of the interaction between TypeVid and the identity of the Video Sheep on time spent with the ears asymmetrical. SI: social isolation, Aggr: aggressive interactions, N: neutral.  $P < 0.05$  when two bars share no common letters,  $P < 0.10$  when two bars share no common letters in brackets. For each group, the median is indicated by the blue dot, the box represents the first and third quartiles, the upper whisker extends to the highest data value within the upper limit and the lower whisker extends to the lowest value within the lower limit. Values noted by an asterisk beyond the whiskers are outliers.

Sheep touched the screen more when the video displayed was SI compared to Aggr ( $F_{2,101.9} = 4.26$ ,  $P = 0.039$ ) and Neutral ( $F_{2,101.9} = 4.26$ ,  $P = 0.006$ ) (Figure 3.6). There was no effect of TypeVid on time spent looking at the screen ( $F_{2,127.7} = 1.53$ ,  $P = 0.220$ , Table 3.4) or on the number of approaches towards screen ( $F_{2,125.4} = 1.87$ ,  $P = 0.138$ , Table 3.4).

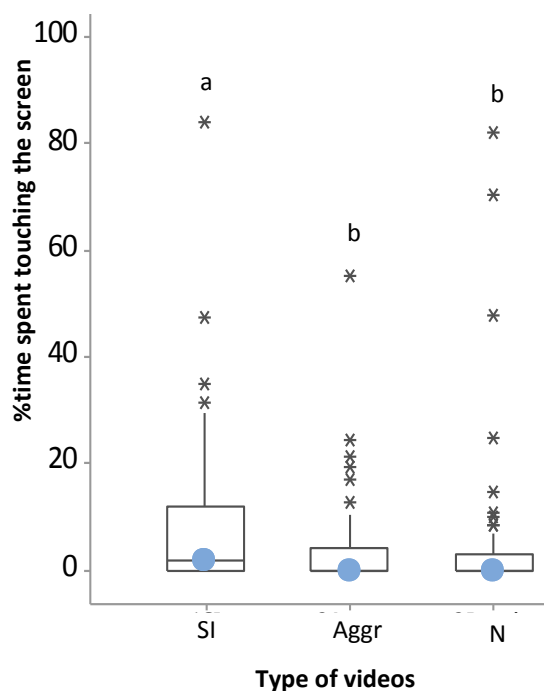


Figure 3.6. Effect of TypeVid on time spent touching the screen.  $P < 0.05$  when two bars share no common letters. For each group, the median is indicated by the blue dot, the box represents the first and third quartiles, the upper whisker extends to the highest data value within the upper limit and the lower whisker extends to the lowest value within the lower limit. Values noted by an asterisk beyond the whiskers are outliers.

PrevVid had no effect on forward ( $F_{3,121.1} = 1.91$ ,  $P = 0.132$ , Table 3.4) and asymmetrical postures (Asymmetrical:  $F_{3,64.5} = 1.05$ ,  $P = 0.377$ , Table 3.4) and was not included in the final fitted model for ears backward. It did not affect time spent looking at the screen either ( $F_{3,138.6} = 1.27$ ,  $P = 0.288$ , Table 3.4). Sheep's approaches of the screen were influenced by PrevVid ( $F_{3,26.5} = 2.90$ ,  $P = 0.054$ ), pairwise comparison showed that sheep approached the screen less when there was no previous video, i.e. during the first session (Table 3.4). There was no difference in approaches between the other types of PrevVid (Table 3.4).

Table 3.4. Effect of TypeVid and PrevVid on time spent in each ear postures, time spent looking at screen and sheep's approaches of the screen. Transformed means and SEM are presented, back-transformed means are presented in brackets to help with interpretation. PrevVid was not included in the final fitted model for Backward ear postures. Significant P values are indicated in bold script.

Variable	Type Video							Previous video							
	Aggr	SI	N	SEM	df	F	P	Aggr	SI	N	None	SEM	df	F	P
Forward	-1.25 (22.0%)	-1.24 (22.2%)	-1.20 (22.9%)	0.17	108.3	0.30	0.74	-1.41 (19.3%)	-1.23 (22.3%)	-1.42 (19.2%)	-0.86 (29.5%)	0.22	121.1	1.91	0.13
Backward	-0.73 (32.3%)	-0.84 (30.0%)	-0.77 (31.5%)	0.17	112.6	0.33	0.72	-	-	-	-	-	-	-	-
Asymm.	-0.59 (35.5%)	-0.61 (35.1%)	-0.51 (37.4%)	0.14	100	0.33	0.72	-0.50 (37.6%)	-0.43 (39.3%)	-0.50 (37.6%)	-0.86 (29.5%)	0.20	64.5	1.05	0.38
Looking at screen	-1.83 (13.5%)	-2.23 (9.3%)	-2.05 (11.0%)	0.23	127.7	1.53	0.22	-2.03	-2.02	-2.35	-1.75	0.29	138.6	1.27	0.29
Approaches	0.13 (0.53)	-0.03 (0.49)	-0.64 (0.34)	0.34	125.4	1.87	0.14	0.56 (0.63)	0.15 (0.54)	-0.05 (0.49)	-1.39 (0.20)	0.55	26.5	2.90	0.05

### 3.3.2 Assessment of affect manipulation treatment

There was no difference between the two housing treatment groups for faecal glucocorticoids concentration (Negative housing:  $101.6 \pm 8.9$  ng/g of faeces; Control:  $109.6 \pm 10.1$  ng/g of faeces;  $F_{1,34} = 0.36$ ,  $P = 0.555$ , Figure 3.7). The two treatments did not differ in their novel object test results either (Looking at NO:  $F_{1,34} = 1.20$ ,  $P = 0.281$ ; Touching NO:  $F_{1,34} = 0.30$ ,  $P = 0.586$ ; Looking at exit door:  $F_{1,34} = 0.72$ ,  $P = 0.403$ ; Looking at other:  $F_{1,34} = 0.02$ ,  $P = 0.895$ ; Table 3.5).

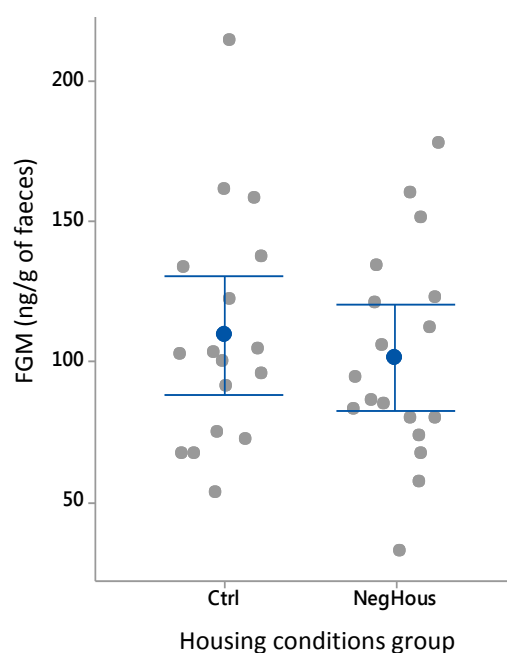


Figure 3.7. Effect of the housing conditions on faecal glucocorticoids concentration (FGM). Each grey dot represents one individual sheep. The mean of each group and 95% CI are indicated by the blue dots and bars.

Table 3.5. Effect of housing conditions on outcome variables from the novel object (NO) test. Transformed means and SEM are presented, back-transformed means are presented in brackets to help with interpretation.

Variable	Control	Negative housing	SEM	df	F	P
Looking at NO	-2.71 (5.8%)	-2.41 (7.8%)	0.192	34	1.20	0.281
Touching NO	-3.27 (3.2%)	-2.96 (4.5%)	0.389	34	0.30	0.586
Looking at exit	1.46 (81.4%)	1.13 (75.9%)	0.271	34	0.72	0.403
Looking others	-2.73 (5.7%)	-2.78 (5.4%)	0.278	34	0.02	0.895



### 3.4 Discussion

We investigated the reactions of sheep to videos of conspecifics filmed in situations of varying valence, and whether mood had an impact on sheep's reactions to these videos, using different housing conditions to induce a low-mood state in the animals. We found differences in the reactions of the sheep to the videos. Housing conditions also affected the reactions of the sheep to the different types of videos

#### Spontaneous reactions to videos

In humans, a 'negativity bias' has been shown, i.e. negative stimuli elicit faster and stronger responses (Ito et al., 1998; Carretié et al., 2001). In a study where sheep were exposed to videos of conspecifics in social situations of varying valence, attentiveness ('head turned towards the screen for at least 2s') was higher for videos of agonistic interactions (negative stimuli), compared to videos of co-feeding (Vögeli et al., 2015). We also concluded from our first experiment that sheep were more attentive towards images of conspecifics in negative situations (Chapter 2). We thus expected sheep to be more attentive towards videos of negative stimuli, and to display more behaviours related to attention (looking at the screen, touching the screen, ears pointed forward in direction of the screen) than when presented with videos of a neutral situation.

We found that sheep spent longer touching the screen when the video displayed was of a conspecific in social isolation (SI) compared to the videos of ruminating sheep but also compared to videos of agonistic interactions. This agrees with our hypothesis of a higher interest towards negative stimuli, and would indicate that the SI videos were indeed perceived as negative stimuli by the sheep, or at least as more interesting than Neutral videos. Nonetheless, it is surprising that stronger differences were not observed between negative and neutral videos. The two negative

situations appear to have been perceived differently, since sheep were more willing to make contact with videos of conspecifics displaying stress behaviour than agonistic behaviour. This makes sense from a biological point of view. Even though aggressive behaviours were not directed towards the sheep watching the video, observing an agonistic interaction and its result from a distance would be a safer strategy. However, if sheep showed more interest for the social isolation videos than for the rumination videos, there was no difference between Neutral and Aggr videos. Animals can provide social support to each other (Rault, 2012). For instance, ewes have been shown to attend more to the lambs showing the strongest pain behaviour after castration (Futro et al., 2015). Another explanation for the higher interest shown in the social isolation videos compared to the others two could thus be linked to this ability for social support.

Two animals interacted on the Aggr videos while only one appeared on the SI videos, and this could be another factor explaining the difference in reactions towards the two types of negative videos. Sheep could be more attracted towards a single conspecific, not engaged in any social interactions, rather than towards a pair of animals. However, the sheep touched the screen for a similar proportion of time when exposed to Neutral videos also showing only one individual compared to when exposed to Aggr videos. The number of familiar conspecifics in the video clip can thus not explain the difference in reaction to SI and Aggr videos. In their study, Vögeli et al. (2015) observed increased attention towards videos of aggressive interactions in comparison to videos of ruminating or feeding sheep. Vögeli et al. suggested that the higher rate of movement recorded (Bovet and Vauclair, 2000) during videos of aggressive interactions could be linked to the increased attention, in combination with the valence of the video stimuli. This is an interesting hypothesis; however, in our videos of social isolation (SI), the Video Sheep were displaying very active behaviour

(e.g. pacing, jumping, escape attempts, head movements) and rates of movement thus appear to have been similar between SI and Aggr videos. However this is an empirical observation, similar to the ones made by Vögeli et al (2015), and could be quantified in more details. Nonetheless, differences in movement rates in the videos can thus not be a satisfactory explanation for our results.

There was no significant difference in time spent in each of the ear postures between the different types of videos, which is surprising considering previous studies where different types of emotional stimuli did affect ear postures in sheep (social situations and feed expectations: Reefmann et al., 2009a; novel and sudden situations: Boissy et al., 2011; pain-related: Guesgen et al., 2016). Interestingly, when sheep were presented with videos of conspecifics in social situations of varying valence in a previous study, no differences in ear postures had been observed either (Vögeli et al., 2015). The nature of the stimuli, i.e. that they were video clips, could thus potentially explain this lack of effect on ear postures. Nonetheless, we did see an effect of the interaction between the type of video shown and housing conditions on ear postures in our study. This agrees with the hypothesis of mood modulation of short-term emotional state and indicates that the fact that the emotional stimuli were videos of conspecifics is not the only explanation for the lack of variation in ear postures.

### Impact of housing conditions and modulation of emotional response

Based on previous studies in sheep (Reefmann et al., 2012; Destrez et al., 2013b), we firstly predicted that the negative housing conditions would induce a low-mood state in sheep. We also expected mood would modulate short-term emotional responses and that sheep from the negative housing condition group would show stronger emotional reactions towards negative stimuli.

The spontaneous reactions of the sheep to the videos were not impacted directly by affect manipulation treatments, but by the interaction of housing conditions and TypeVid, which means that mood and emotional stimuli did interact. Housing conditions modulated reactions to videos of aggressive interactions. When exposed to Aggr videos, sheep from the negative housing conditions spent less time with the ears forward than sheep from the control group. This agrees with observations from Vögeli et al (2015), where sheep exposed to unpredictable, stimulus-poor housing conditions displayed fewer ears forward compared to sheep from predictable, stimulus-rich conditions. The two housing conditions groups did not differ in their proportions of backward ear postures, contrary to what was observed by Vögeli et al (2015). It is however interesting to note that backward ear postures were affected by the video stimuli in sheep from the negative housing conditions group, but not in sheep from the control group. Sheep from the negative housing group displayed higher proportions of ears backward when looking at Aggr videos compared to SI or Neutral videos. It can also be reported, for general interest, that the opposite pattern was observed for forward ear postures (i.e. lower proportion of ears forward when Aggr shown), but this difference was not statistically significant.

Backward ear postures have been associated with the individual experience of negative situations such as pain (Guesgen et al., 2016), fear (Boissy et al., 2011) or social isolation (Briefer et al., 2015). Higher proportions of ears forward have been reported in situations where a high level of attention is required. Most of these situations are considered to be of negative valence too, such as exposure to an unfamiliar test situation involving mild pain in sheep (Stubsjøen et al., 2009) or separation from group members in sheep (Reefmann et al., 2009a) but this is not always the case (novel odour test in wild mice: Lecorps and Féron, 2015). In goats, the proportion of ears forward increased in situations of high arousal, regardless of the

valence of the situation (Briefer et al., 2015). This is why we consider forward ear postures to be an indicator of interest and increased attention towards a stimulus, rather than an indicator of emotional valence, while backward ear postures have been more clearly linked to negative emotional state in animals. Our results thus suggest that Aggr videos elicited more attention (higher proportion of ears forward), and potentially a more negative emotional state (tendency for a higher proportion of ears backward) in sheep from the negative housing conditions group than in sheep from the control group. Sheep are frequently exposed to agonistic behaviours within the social group; seeing a conspecific in a situation such as social isolation, is less common or impossible. Due to the restriction on feed space, sheep from the negative housing condition group were even more likely to be exposed to aggressive interactions than sheep from the control group. It is thus possible that the social dimension of the negative situation displayed on the video influenced the reactions of the sheep. Since sheep from the negative housing conditions had been exposed to higher levels and frequency of aggressive interactions, being presented with videos of more aggressive interactions could have elicited a stronger negative emotional state in the test sheep from the negative housing conditions. The stronger negative reactions towards videos of negative interactions could also be consistent with the negative cognitive bias observed in anxious and depressed patients who react more negatively to negative stimuli (Hale, 1998; Steger and Kashdan, 2009). From that perspective, the interaction between housing conditions and type of videos would support the hypothesis that the negative housing conditions elicit low-mood state in sheep.

Even though the different housing conditions had an impact on the reactions of the sheep to videos, there was no difference that we could detect between the two groups for faecal glucocorticoids and in the novel object test. The relationship between cortisol and chronic stress is complex (Palme, 2012), and results differ between

studies. For instance, after 9 weeks of exposure to unpredictable negative housing conditions, no differences were found in baseline blood cortisol levels between treatment and control group in sheep (Destrez et al., 2013b). In another sheep study involving 4 weeks of negative housing conditions, blood cortisol levels decreased within the treatment group, while there was no difference before or after the treatment between control and negative housing conditions group (Doyle et al., 2011). From that perspective, comparing faecal glucocorticoids levels before and after the affect manipulation would probably have been more sensitive to the impact of the manipulation on stress physiology. This would also have allowed to deal with individual differences in cortisol levels, and to identify variation patterns.

Since housing conditions had an impact on the sheep reactions to the videos, it is unlikely that the housing conditions did not affect the animal at all. Nonetheless, it is surprising that no behavioural or physiological differences could be detected. Behavioural and physiological assessment of the impact of the housing conditions took place at the end of the treatment period, and after the tests. It is possible that human stimulation, potential anticipation of the hay available in the waiting pens (even though sheep had access to *ad libitum* hay in their home pen too) and exposure to novel stimuli (the videos were different for each session) might have acted as enrichment for the negative housing group and have had a positive effect on the animals. The novel object test took place after four days of tests with videos. Sheep from the negative group had not been exposed to unpredictable negative events anymore during these four days, but restrictions on feed space and pen size remained. The absence of unpredictable events might have reduced differences between the two groups. Consequently, if the novel object test had taken place before the videos were presented, we might have been able to observe more differences between the two groups.

It is also possible that the differences between the two housing treatment were not strong enough. Housing conditions for the control group, despite being above DEFRA's recommendation for pen and feed space allowance, were not otherwise enriched. Housing conditions for the control group could thus have been perceived as negative too, in which case the negative housing conditions would only have been more negative, and the conditions compared would have been quite similar. However, the fact that differences were identified in the sheep reactions to the videos, combined to the high welfare standard of the control housing conditions makes this later hypothesis less likely. Finally, it is worth noting that based on personal observations during tests, the two groups appeared to display different behaviours. Sheep from the negative housing conditions group were subdued and easy to handle, while sheep from the control group were more agitated, and required firmer handling. Unfortunately these behaviours were not quantified and reported. Using handling scores as well as human-animal relationship tests (e.g. approach tests (Forkman et al., 2007)) would have been a useful addition to this study.

### Other factors impacting reactions of the sheep to video stimuli

As suggested by results from our first experiment, the identity of the Video Sheep did affect the sheep's reactions to the videos. When exposed to SI videos, sheep displayed more asymmetrical ears when the Video Sheep was Sheep1 compared to Sheep2. As discussed in the previous chapter, the relationship between weight and dominance is well-established in ungulates (Landaeta-Hernández et al., 2013). Video Sheep were therefore chosen so that they were of average weight in order to limit the risks of choosing an animal with a very high rank in the flock hierarchy. Nonetheless, gathering more information about dominance status (Rioja-Lang et al., 2012) but also affinity relationships (Sibbald et al., 2005) between Video Sheep and test sheep prior to the presentation of the videos could be an improvement in future studies.

PrevVid was included in the analysis to evaluate a potential carry-over effect of the videos. We found that the only effect of PrevVid was due to the very first session: when sheep were exposed to the videos for the first time, they approached the screen less than during any of the following sessions. Novelty is a known fear-eliciting stimulus in sheep (Vandenheede and Bouissou, 1994; Boissy and Erhard, 2014) and the first exposure to a video stimulus could thus have been frightening. After that first session it is likely that sheep habituated to the moving videos displayed and so were less afraid to investigate the screen. Despite seeing the screen lit and displaying a video of an empty pen during the habituation phase, sheep still perceived the presentation of videos as novel and potentially fear-eliciting. Including a sham test session, with the presentation of a video of a moving item during the habituation phase might have reduced this effect of the first session.

### 3.5 Conclusion

There was an effect of the interaction between housing conditions and the type of video shown. Aggressive interactions elicited a more negative response. Housing conditions had an effect on sheep spontaneous reactions to the videos, and sheep from the negative housing conditions reacted more strongly and more negatively to negative videos, especially of aggressive interactions. This study support previous results showing that negative housing conditions induce a low-mood state in sheep that influences their responses to emotional stimuli. However there was no detectable influence on housing conditions on other behavioural and physiological measures. Our results indicate that sheep can perceive differences in the valence of emotional stimuli from conspecifics and that their perception of emotions is influenced by housing conditions.



## Chapter 4: Face-based perception of emotions in dairy goats

## Abstract

Faces of conspecifics convey information about identity, but also gaze, and attentional or emotional state. As a cognitive process, face-based emotion recognition can be subject to judgment bias. In this study we investigated whether dairy goats ( $n=32$ ) would show different responses to 2-D images of faces of familiar conspecifics displaying positive or negative emotional states. We also examined the possible use of images of faces as stimuli in cognitive bias studies. The faces of four subjects were photographed in a positive and a negative situation. Three types of images of ambiguous facial expressions were then created using morphing software (75% positive, 50% positive, and 25% positive). In a test-pen, each goat was exposed for 3 seconds to each type of image, obtained from the same goat. All goats were shown non-morphed faces first, before being shown the three types of morphed faces, balanced for order. Finally, the first non-morphed face was shown again. Spontaneous behavioural reactions including ear postures (forward, backward and asymmetrical) and interactions with the screen (time spent looking or touching) were recorded during the 3 seconds. Results were analysed using REML with repeated measurements. Goats spent more time with their ears forward when the negative image was shown compared to the positive ( $F_{4,121.3} = 2.51$ ,  $P = 0.018$ ), indicating greater interest in negative faces. Identity of the photographed goat influenced the time spent with the ears forward ( $F_{2,57.4} = 7.01$ ,  $P = 0.002$ ). We conclude that goats react differently to images of faces displaying different emotional states and that they seem to perceive the emotional valence expressed in these images. Response to morphed faces was not necessarily intermediate to response to negative and positive faces, and not on a continuum. Further study is thus needed to clarify the potential use of faces in cognitive bias studies.

## 4.1 Introduction

It is now generally accepted both in the scientific community and by policy makers that animals are sentient beings, capable of experiencing emotions (de Vere and Kuczaj, 2016). Being able to assess emotional states in farm animals is crucial to improving their welfare. Emotions are defined as short-term internal psychological states induced by external or internal stimuli (Désiré et al., 2002). According to Dantzer (2002) an emotional state has behavioural (e.g. running away from a frightening stimulus), physiological component (e.g. increase of heart-rate) and subjective (e.g. 'I feel frightened') components. Evidence of behavioural and physiological components of emotions has been shown repeatedly in animals (Désiré et al., 2002). The subjective dimension of emotions is of course difficult to evaluate in animals, since there can be no use of language for self-report as in psychology. However the development of methodologies such as judgement bias or attention bias tests in animals can give the researcher an indirect access to the subjective dimension of emotions in animals (Roelofs et al., 2016b). An emotion can also be characterised by a combination of its valence, i.e. positive vs. negative, and its arousal, i.e. low or high. For example, fear has a negative valence and a high level of arousal (Mendl et al., 2010).

Although the function of emotion is not primarily for communication, the outward expression of an emotional state involves changes in posture, vocalisations, odours and facial expressions, which can be perceived and used as indicators of emotional state by other animals (Terlouw et al., 1998; Siniscalchi et al., 2013). Since conspecifics can perceive one another's emotions, understanding how emotions are identified and how they can spread within a social group could have a major impact on improving the welfare of farmed species that are reared in groups. This study was a step in that direction and focused on face-based emotion recognition in goats, a highly

social livestock species with complex hierarchical relationships (Miranda-de la Lama and Mattiello, 2010) . The fact that the facial expressions of humans and nonhuman mammals have a lot in common was suggested first by Darwin (1872). For social species, faces are a major source of information (Little et al., 2011); features that allow the identification of the individual, but also the direction of gaze, attentional state and emotional state are conveyed through the face (Adolphs, 2002). Face perception, and more specifically the processing of emotions, has been widely studied in sheep, which can discriminate between calm and stressed faces of conspecifics and humans in 2-D images (Tate et al., 2006).

As small ruminants, goats are closely related to sheep. Goats also have excellent visual acuity (Blakeman and Friend, 1986), and have been shown to use visual social signals (Kaminski et al., 2005). They live in complex social groups, with non-linear hierarchies (Collias, 1956; O'Brien, 1988). We therefore hypothesised that face-based perception of emotions in goats would be as developed as in sheep. Since goats display behavioural expressions that differ between situations of positive and negative valence (Briefer et al., 2015), we wished to determine if those displays would impact the goats' faces sufficiently so that a difference could be perceived by conspecifics. We therefore tested whether goats would react differently to 2-D images of faces of familiar conspecifics displaying positive or negative emotional states. The images used were obtained by filming goats during two types of interactions with a human handler. We also hypothesised that goats would display behaviours indicating negative valence when looking at negative faces, and positive valence when looking at positive ones.

Recent studies demonstrated that the emotional state of an animal can influence cognitive processes, such as learning, attention or judgement (Mendl et al., 2009). Judgement bias tests have been used in farm animals to assess emotional states,

especially after manipulation of the environment to induce positive or negative emotional states or as a tool to assess the impact of husbandry practices (reviewed by Baciadonna and McElligott, 2015). Animals in a negative emotional state show pessimistic judgements (i.e., react in a similar way to negative and ambiguous stimuli) while those in a positive emotional state make optimistic judgements about ambiguous stimuli (i.e., react in a similar way to positive and ambiguous stimuli). Face-based perception of emotion is a cognitive process (Martin et al., 2012) and as such is potentially subject to this judgement bias. To test if images of faces could be used as cognitive bias stimuli, we produced three types of ambiguous faces ranging in valence from negative to positive, using morphing software. For these images to be usable in cognitive bias studies, goats have to show distinct spontaneous reactions to images of goat faces taken in positive or negative situations. Furthermore goats have to show gradual intermediate responses to the morphed faces to comply with the cognitive bias response pattern.

Finally, since goats were exposed repeatedly and without reinforcement to the same type of stimuli, we wanted to test their level of attention after five exposures, and thus included a final test session that was a repeat of the first.

## 4.2 Methods

### 4.2.1 Ethical note

All experimental procedures were approved by the Animal Welfare Advisory Board of the research unit (INRA) and complied with the recommendations of the GRICE (Groupe de réflexion interprofessionnel sur les comités d'éthique appliquée à l'expérimentation animale, or Interprofessional reflexion group on ethics committees applied to animal experimentation) recommendations. Animals were weighed weekly

before, during and after the study, and were subjected to regular veterinary examinations as part of the farm schedule. No animals had to be removed from the study due to illness or injury.

#### 4.2.2 Animals and management

The experimental work took place between April and May 2015 at the INRA experimental farm at Thiverval-Grignon, France. 32 lactating Saanen ( $n=17$ ) and Alpine ( $n=15$ ) goats aged 18 months were used in this experiment. The animals had been removed from their dams after birth and artificially reared in mixed-breed groups. They were all familiar with each other, having lived in the same group for at least six months prior to the trial.

The 32 goats were tested in two groups of 16, balanced for breed (Group 1: 8 Alpine and 8 Saanen ; Group 2: 7 Alpine and 9 Saanen) and weight (Group 1:  $55.3 \pm 6.5$  kg ; Group 2:  $53.7 \pm 5.2$  kg). For the duration of the study, goats from both groups were housed together in the same straw pen that was set within the main farm building ( $1.25 \text{ m}^2$  per goat). Morning milking took place between 07.30 and 09.30, and afternoon milking between 15.30 and 17.30. The goats were fed a standard ration (mixed ration of hay, soy cake, barley and dehydrated alfalfa) twice a day ad libitum. Goats had unlimited access to water.

For each group the tests were completed in four days. Two days separated the trials for Group 1 and Group 2.

#### 4.2.3 Images of faces

Amongst the 32 goats, two Saanen and two Alpine were selected to be filmed to produce images of faces. The choice of the filmed animals, hereafter referred to as

Photo Goats, was based on their individual reactions to humans. The positive situation consisted of a positive interaction with an experimenter, so choosing animals with a good human-animal relationship was essential. To select the Photo Goats, a familiar experimenter entered the home pen and stood immobile, without speaking. The first two goats of each breed to approach the experimenter of their own volition in the home pen were selected to be the Photo Goats. To produce the images, each Photo Goat was placed into two different situations that were likely to elicit a positive and a negative emotional state respectively. Rewarding stimuli are thought to elicit positive emotional states, while punishing stimuli (predator, pain, stress) elicit negative emotional states (Mendl et al., 2010). Behavioural observations were used in conjunction with this framework to determine the valence of the situation the goats were placed in.

Photo Goat faces were filmed with a HD camera (HDR-XR155, Sony, Japan). Frames with a full clear frontal view of the face were extracted from those short video clips using Pinnacle Studio 17 (Pinnacle Systems, 2013). The faces were then digitally cut from the frames and placed against a neutral beige background (RGB model: R=217, G=202, B=126) with Adobe Photoshop CC (Adobe Systems, 2014) to create the images used in the tests (Figure 4.1).

#### 4.2.3.1 Positive situation

The Photo Goats were groomed by a familiar experimenter in the home pen. Pleasant grooming consisted of gentle scratching of the neck and shoulder areas for approximately 5 minutes. Grooming of this sort has been shown to be a gentle interaction in cattle (Schmied et al., 2008) and to induce a positive judgment bias in goats (Baciadonna et al., 2016). Since the Photo Goats had been chosen based on the fact that they voluntarily approached humans, habituation was not necessary. During

grooming the Photo Goats did not move away and after grooming they repeatedly sought attention from the experimenter. These observations supported the idea that grooming was pleasurable and thus rewarding and induced an emotional state of low arousal and positive valence (Coulon et al., 2015). Goats had their ears lowered and turned down during almost the entire grooming session, and pictures of the animal displaying this ear posture were extracted from the videos. These images are hereafter named the positive images (Figure 4.1).

#### 4.2.3.2 Negative situation

Each Photo Goat was isolated in a weigh-crate, located within the main building, thus allowing continued auditory and olfactory contact with other goats. The negative stimulus was produced by an experimenter who applied an ice block to the udder for a maximum of 30 seconds, or until a negative reaction from the goat (e.g. stamping, sharp head movements, trying to leave the crate) was observed. The obvious thermal discomfort induced by the application of the ice pack made the application of ice to the udder a punishing situation. This was highlighted by attempts made by the Photo Goat to escape the source of discomfort and the situation was thus considered to have induced a negative state of high arousal. As soon as a good quality video was captured the goat was brought back to the group. All Photo Goats displayed a negative reaction and avoidance behaviours when the ice block was applied, which, according to Mendl et al's framework (2010) suggests that it did elicit an emotional state of high arousal and negative valence.

Pictures from the first reaction of the goat to the ice block were extracted from the films, when the animal raised its head, with the tip of the ears pointing backward and the auricles turned downwards. These images are hereafter named the negative images (Figure 4.1).



### 4.2.3.3 Morphed faces

The use of morphed images allowed the creation of intermediate images that were 25% (I-), 50% (I50) and 75% (I+) between the negative and positive images (Figure 4.1).

Intermediate stimuli of each Photo Goat were produced by morphing images obtained in a negative and in a positive situation from the same goat (WinMorph 3.01, DebugMode, 2012). Key facial-features such as eyes, nostrils, mouth, ears and shape of the forehead and the jaw were marked on the positive and negative faces (Figure 4.1a). The positive face was then distorted by the software into the negative one frame by frame.

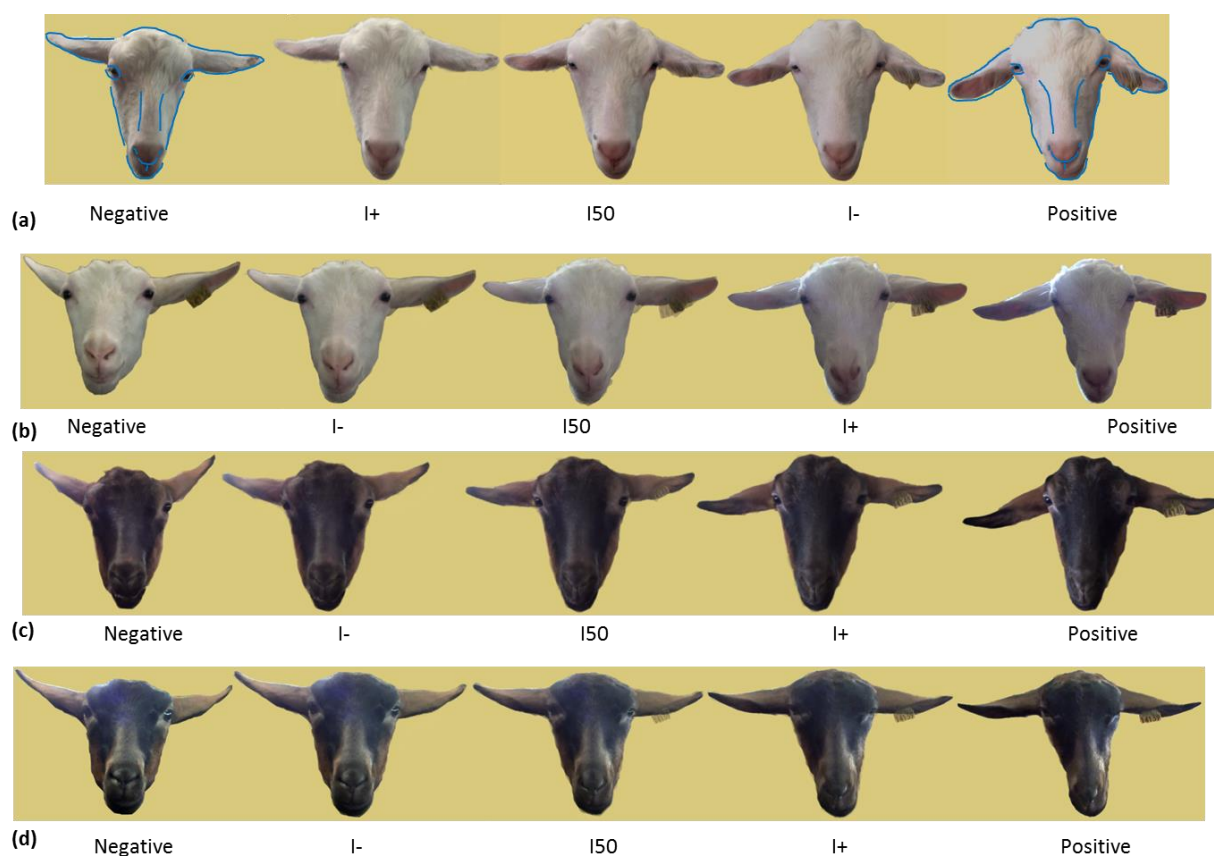


Figure 4.1. The five types of images of faces obtained from four different goats: (a) Photo Goat Saanen 1 (b) Photo Goat Saanen 2, (c) Photo Goat Alpine 1, (d) Photo Goat Alpine 2. 'Negative' images were taken when an icepack was applied to the udder. 'Positive' images were taken

while the goat was being groomed by a familiar experimenter. The three types of images of ambiguous facial expressions were created using morphing software (25% positive (I-), 50% positive (I50), and 75% positive (I+)). The blue lines (a) outline key facial-features marked on the positive and negative faces in the morphing software.

#### 4.2.4 Tests: spontaneous reactions to images of faces

##### 4.2.4.1 Test pen

The test pen was located outside the main building in a covered area approximately 40m away from the home pen (Figure 4.2). The waiting pen was adjacent to the test pen but separated from it by a wall of straw bales. The test pen had solid wooden walls. An extra wooden panel prevented entry to one corner of the test pen and prevented goats from standing in the blind spot of the cameras. A computer screen (19 inch, Dell) was placed on the wall opposite the entrance at eye-level for goats, to display images of faces. The screen was not present during the habituation period. There was a small opening in the solid wall above the screen. During test sessions, the experimenter could place small items through the hole to draw the attention of the goat to the computer screen, in a standardised way described in Session 4.2.4.1.

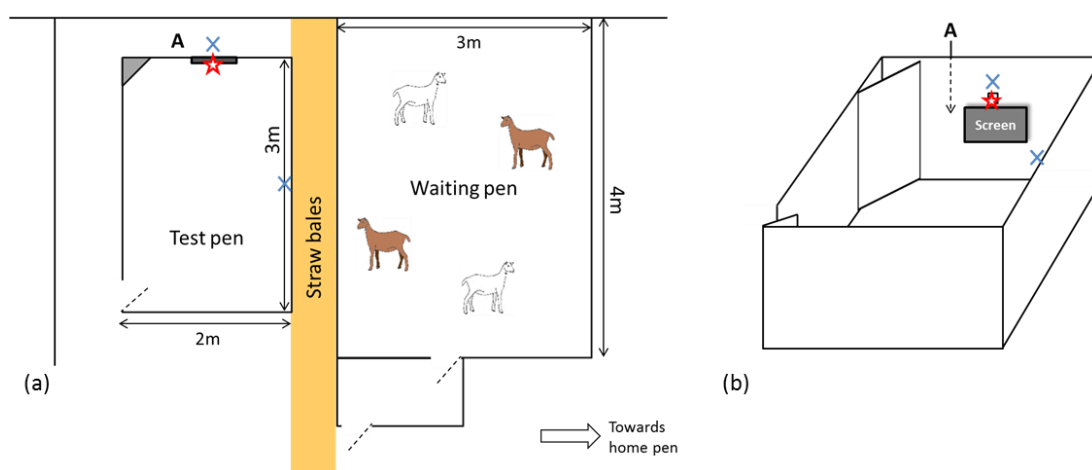


Figure 4.2. (a) Schematic representation of the test and waiting pens. (b) 3D view of the test pen. 'A' indicates the location of a hidden experimenter responsible for catching the goat's

attention and the red star where the items were moved above the screen to catch the goat's attention. Blue crosses represent the two cameras.

#### 4.2.4.2 Habituation

Goats were habituated to the experimental set-up prior to the beginning of the tests session. The habituation day was divided into three sessions. In the first session, the goats were brought into the test-pen in pairs once for 5 minutes. In the following two sessions, they were brought into the test-pen alone for 2 minutes. Goats were considered habituated to the experimental set-up since they walked in calmly and entered the test arena easily.

#### 4.2.4.3 Test sessions

The three days of testing followed the habituation. Order of testing was balanced for breed and 'identity of the Photo Goat displayed' as well as for the type of image presented as far as possible. The order of testing of the goats was the same in all sessions. Each goat was shown the images of one Photo Goat of its own breed, resulting in the images of each Photo Goat being presented to a total of eight other goats. The Photo Goats were tested as well, and presented with images of the other Photo Goat of their breed and not themselves.

Goats (including the Photo Goats) were exposed to one image per test session, with two sessions per day and a total of six sessions across three days, with a different image shown in each session. For Sessions 1 and 2, the images shown were always the real positive and negative images, to obtain a baseline of the goats' reaction to images of real faces to allow comparison of the two emotions. In each group, for Session 1 half of the goats were exposed to the positive image and the other half to the negative. For Session 2, the goats were exposed to the second type of real image compared to Session 1. During Sessions 3, 4 and 5, the goats were exposed to the morphed images (I+, I50, I-

). The order of testing of each morphed face was balanced so that in each session the same number of goats saw a given morphed face. Session 6 was a repetition of Session 1, and was used to test if the goats were still paying a similar level of attention to the image and if they were still reacting to the image.

One hour after morning milking, 16 goats were brought in pairs and on a leash to the waiting pen. The goats were not always brought in the same order, and it took approximately 15 minutes to bring all goats to the waiting pen. Once all goats had been brought to the waiting pen, they were given 15 minutes to settle down before the first tested goat was taken on a leash to the test pen. The test started when the door of the test pen was locked behind the goat.

Before showing an image on the screen, we wanted to be certain that the goat was directing its attention towards it, and so that it would react to the stimulus presented only. This is why images of faces were not already displayed when the animals entered, but only as soon as the goat paid attention to the (dark) screen. A goat fulfilled the 'paying attention' criterion when its head was oriented towards the screen for at least 1 second. To direct the attention of the goat towards the screen, an experimenter hidden behind the screen (position A) waved items through the opening made above the dark screen at the start of each session (Figure 4.2). The experimenter switched to a different item every ten seconds until the goat reached the 'paying attention' criterion, and the order of presentation of the items was the same for every goat. If more than three different items had been used unsuccessfully, the hidden experimenter started speaking to attract the goat's attention, using standardised sentences and sounds. The experimenter tried to catch the goat's attention until the goat fulfilled the criterion. There was no time limit, and it took 32 seconds on average, ranging from 0.2 to 216 seconds. Once this occurred, an image of a Photo Goat's face

was displayed on the screen for 3 seconds after which the screen went dark again. The 3 seconds presentation duration can be considered to be a very short interval. However emotions are by definition of short duration (Désiré et al., 2002), and facial displays of emotions are brief and typically last between 0.5 and 2 sec in humans (Shreve et al., 2009). Presenting facial expressions for a very short time is thus closer to mimicking real life situations. In human neuroimaging studies of face-based emotion recognition, stimuli are presented for similarly short periods of time,  $\leq 3$  sec (Lee et al., 2008a). We also chose a presentation length of 3 seconds because we were only interested in the spontaneous reactions of the goats to the images. Limiting habituation to the presentation of images was also key due to the repeated exposures, and a very short exposure to the stimuli helped to preserve the goat's relative naivety towards images of faces.

The behaviour of the goat was video recorded from the start of the test session until the image disappeared. The animal was then returned to the waiting pen and the next goat brought for testing. Forty-five minutes after the last goat was tested in the first session of the day, the first was tested again to start the second session, resulting in an interval of approximately two hours between sessions for each goat.

#### 4.2.5 Data collection and analysis

Behaviours described in Table 4.1 were scored from the video recording for each test session using The Observer 5.0 (Noldus Information Technology, Netherlands). The observer was blind to the type of image shown. Due to the very short duration of observations, video playback speed was slowed down by a factor of 10 for behavioural observations, and so every change in ear postures was recorded.

The outcome variables were percentages of time spent with the ears forward, backward, asymmetrical, and the percentage of time spent interacting with the screen (oriented towards and/or touching). Horizontal ear postures did not occur, so this behaviour was not included in the analyses. Time spent with the ears in forward, backward or asymmetrical postures summed to the total duration of the observation (i.e., sum-one constraint). Outcome variables were logit-transformed to conform to assumptions of the normality and homogeneity of the data. The predictor variable latency before the goat reached the 'attention OK' criterion (LatCrit) was ln-transformed for the same reasons. Time spent with the ears asymmetrical could not be transformed to conform with normality assumptions, and was thus transformed into a binomial variable (1 = asymmetrical ears occurred, 0 = asymmetrical ears did not occur).

All outcome variables were analysed for the 3 seconds interval when the image was displayed. Analyses were conducted in GenStat 16<sup>th</sup> edition (VSN International Ltd., United Kingdom). The significance level was set at  $P=0.05$ . All data in the text are presented as means  $\pm$  1 standard deviation, unless otherwise stated. We first compared spontaneous reactions to all five types of images, taking into account all six sessions.

Table 4.1. Recorded behaviours and transformation applied to outcome variables in goats when shown images of familiar conspecifics on a screen for 3 sec. Ears postures were adapted from Briefer et al, 2015.

Behaviour		Description	Unit	Transformation	Type
<b>Beginning of the test</b>		Once the door of the test pen was locked behind the goat	---		---
<b>Latency to pay attention to the screen</b>		Latency between the beginning of the test and the display of the image on the screen	sec (0.2 to 216 sec)	Ln	Predictor
<b>Distance to the screen when image displayed</b>		Estimated distance between the tip of the nose and the screen, when the image appears on the screen	6 categories from 50 – 300 cm	---	Predictor
<b>Interacting with the screen</b>		Time spent with the 2 eyes and the head in direction of the screen, regardless of the direction of the body (“looking”) or touching the screen (nose or lips touching the screen) while the image is displayed on the screen	sec (0.2 to 3 sec)	Logit	Outcome
<b>Ear postures</b>	<b>Ears forward</b>	Tip of both ears pointing towards the front of the goat	sec (0 to 3 sec)	Logit	Outcome
	<b>Ears backward</b>	Tip of both ears pointing towards the back of the goat	sec (0 to 3 sec)	Logit	Outcome
	<b>Ears asymmetrical</b>	Right and left ears in different position regarding a perpendicular to the head-rump axis	sec (0 to 3 sec)	Logit	Outcome
	<b>Ears horizontal</b>	Ears in a central posture, along a perpendicular to the head-rump axis	sec (0 to 3 sec)	Logit	Outcome

Continuous data were analysed by linear mixed models (REML) with repeated measurements. A power model for covariance was used to account for correlations within subjects across time. Power models allow unevenly spaced time points to be taken into account (e.g. that Sessions 1 and 2 were on the same day and thus closer in time than Sessions 2 and 3), since the correlations between measurements decrease as the time between two time points increase. Correlations between time points will thus be larger Sessions 1 and 2 than for Sessions 2 and 3, even though they are all consecutive sessions. Heterogeneity of variance across test sessions was allowed when it led to a smaller deviance of the model (one-tailed test with a  $\chi^2$  distribution). The occurrence of the asymmetrical ear postures was analysed by general linear mixed model (GLMM) with a binomial distribution and logit link function. Breed, the type of image shown during the test session (TypeImage), Identity of the Photo Goat, the type of image shown during the previous session (PrevIm), DistScreen and LatCrit were considered as potential fixed effects. The interaction between TypeImage and Identity of the Photo Goat was also included in the list of potential fixed effects, since it was the most biologically relevant interaction in our design. Fixed effects were then fitted by stepwise backward selection for each outcome variable, and not all fixed effects listed above were included in the final model for each variable (see Table 4.2 for a detailed description of the fixed effects considered simultaneously in the final models). When a predictor was not included in the final fitted model, no statistical results are presented for that predictor. Session and Animal were included as random effects as Animal nested within Session. Post-hoc analyses were conducted using Fisher's Least Significant Difference tests. Data from Sessions 1 and 6 were also analysed separately following the same method, to compare the responses of the goats to the same stimuli presented twice.

Table 4.2. Final fixed effects fitted by stepwise backward selection for each outcome variables (forward, backward and asymmetrical ear postures and time spent interacting with the screen)



Variable	Fitted fixed effects
<b>Forward</b>	Breed + Typelm <sup>1</sup> + iPG <sup>2</sup> + PrevIm <sup>3</sup> + DistScreen <sup>5</sup> + LatCrit <sup>6</sup>
<b>Backward</b>	Breed + Typelm <sup>1</sup> + iPG <sup>2</sup> + PrevIm <sup>3</sup> + DistScreen <sup>5</sup>
<b>Asymm.</b>	Breed + Typelm <sup>1</sup> + iPG <sup>2</sup> + Typelm*iPG + DistScreen <sup>5</sup> + LatCrit <sup>6</sup>
<b>Interacting</b>	Breed + Typelm <sup>1</sup> + iPG <sup>2</sup> + Typelm.iPG + PrevIm <sup>3</sup> + DistScreen <sup>5</sup>

<sup>1</sup>Typelm = Type of image displayed on the screen, could be positive, negative, I+ (75% positive), I50 (50% positive), and I- (25% positive)

<sup>2</sup>iPG = identity of the goat displayed on the screen (Photo Goat)

<sup>3</sup>PrevIm = type of previous image shown

<sup>5</sup> DistScreen = Distance in cm between the head of the goat and the screen when the photo appeared

<sup>6</sup> LatCrit = latency before the goat reached the 'paying attention' criterion, i.e. stared at the screen for at least 1 sec

## 4.3 Results

### 4.3.1 Spontaneous reactions to different types of images

Goats reacted differently to different images of faces (Positive, I+, I50, I- and Negative). TypeImage had a significant effect on forward ear postures ( $F_{4,121.3} = 2.51$ ,  $P = 0.045$ ). Post-hoc comparisons showed that goats spent significantly more time with their ears forward when the negative image was shown compared to the positive ( $F_{4,121.3} = 2.51$ ,  $P = 0.018$ ) and to I- images ( $F_{4,121.3} = 2.51$ ,  $P = 0.011$ ) (Figure 4.3a). There was no significant effect of TypeImage on time spent with the ears backward ( $F_{4,139.4} = 1.73$ ,  $P = 0.147$ , Figure 4.3a) or on the occurrence of asymmetrical ears ( $F_{4,53.9} = 0.34$ ,  $P = 0.850$ , Figure 4.3a). The interaction between TypeImage and the identity of the Photo Goat had an effect on time spent interacting with the screen ( $F_{12,73} = 3.65$ ,  $P < 0.001$ , see Figure 4.5 and Table 4.3).

Identity of the Photo Goat had an effect on the time spent with the ears forward ( $F_{2,57.4} = 7.01$ ,  $P = 0.002$ ) but not on the time spent with the ears backward ( $F_{2,29.6} = 1.35$ ,  $P = 0.274$ , Figure 4.3b) or on the occurrence of asymmetrical ears ( $F_{2,26.8} = 0.10$ ,  $P =$

0.905, Figure 4.3b). Thus, goats exposed to images taken from Photo Goat 'Saanen 2' spent more time with their ears forward (Figure 4.3b) regardless of the type of image shown. Conversely, goats that looked at images taken from 'Alpine 2' spent less time with their ears forward (Figure 4.3b) compared to Alpine 1 and Saanen 2 and more time compared to from 'Saanen 1', regardless of the type of image shown.

The distance between the goats and the screen when the image appeared (DistScreen) also affected the goats' ears postures in reaction to images (Figure 4.4). Regardless of the type of image shown, if a goat stood at a distance of more than 50 cm for the screen when the image appeared, it spent more time with its ears forward ( $F_{5,145.4} = 10.22$ ,  $P < 0.001$ ). In contrast, the closer a goat stood to the screen, the more time it spent with the ears backward ( $F_{5,165.3} = 7.89$ ,  $P < 0.001$ , Figure 4.4) and the more asymmetrical ear postures it displayed ( $F_{5,143.4} = 2.7$ ,  $P = 0.019$ , Figure 4.4).

Finally, Alpine goats spent longer interacting with the screen than Saanen goats ( $F_{1,66.5} = 4.39$ ,  $P = 0.040$ ; Alpine:  $75 \pm 29\%$ , Saanen:  $68 \pm 37\%$ ). There was no effect of breed on any of the other outcome variables (Ears Forward:  $F_{1,54.8} = 0.04$ ,  $P = 0.836$ ; Ears backward:  $F_{1,28.4} = 0.14$ ,  $P = 0.706$ ; Ears asymmetrical:  $F_{1,28.2} = 0.02$ ,  $P = 0.793$ ).

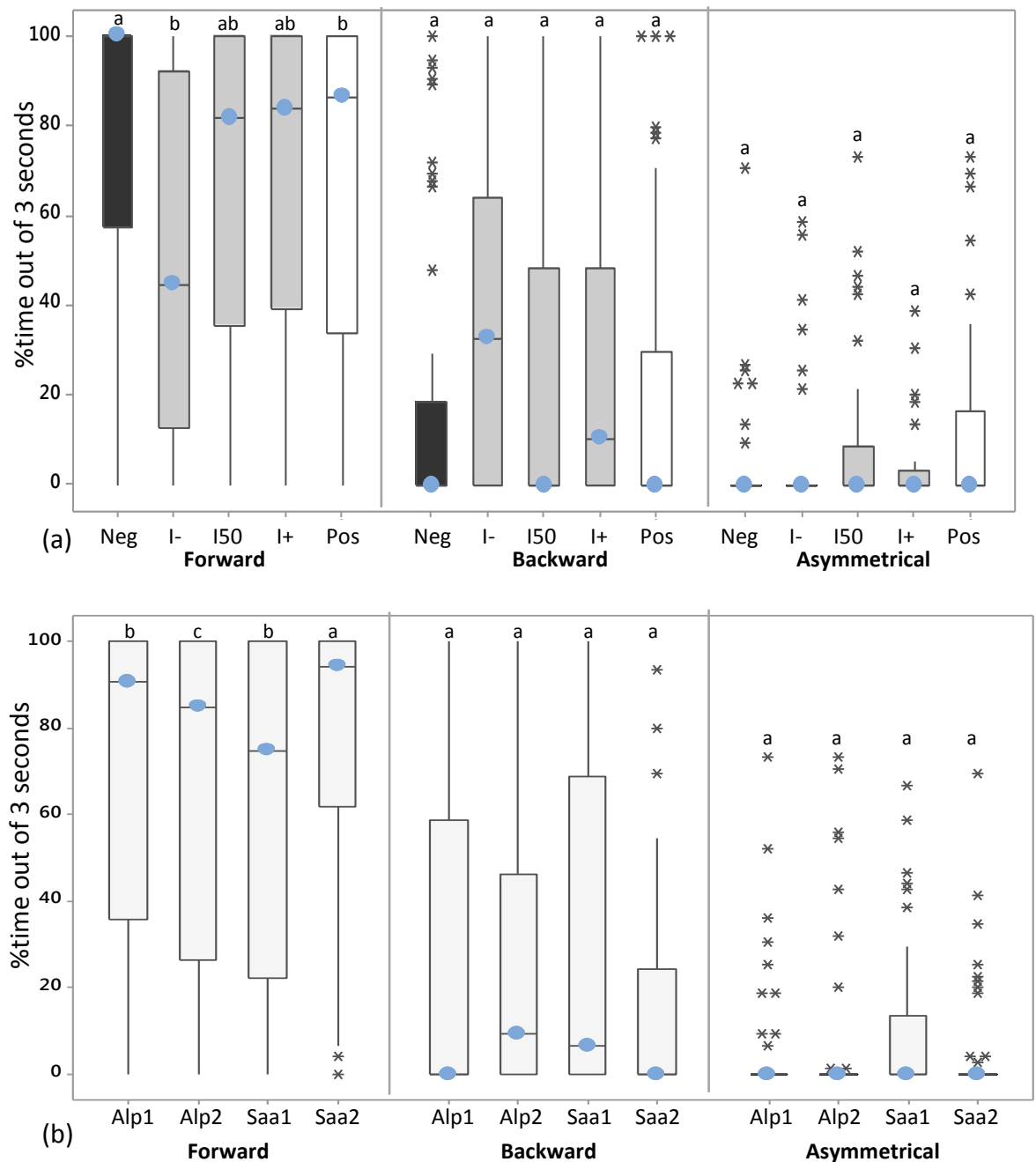


Figure 4.3. Effect of the type of images (a) and of the identity of the goat on the image (b) on the percentage of time spent in different ear postures in goats when shown different types of images of faces of familiar conspecifics on a screen for 3 sec. Five images of the same goat of its own breed (Alpine (Alp) or Saanen (Saa)) were shown to any given goat. 'Negative': image taken while an icepack was applied to the goat udder. 'Positive': image taken while the goat was being groomed by a familiar experimenter. The three other types of images were of ambiguous facial expressions created using morphing software (25% positive (I-), 50% positive (I50), and 75% positive (I+)).  $P < 0.05$  when the bars share no common letters. For each group, the median is indicated by the blue dot, the box represents the first and third quartiles, the upper whisker extends to the highest data value within the upper limit and the lower whisker extends to the lowest value within the lower limit. Values noted by an asterisk beyond the whiskers are outliers.

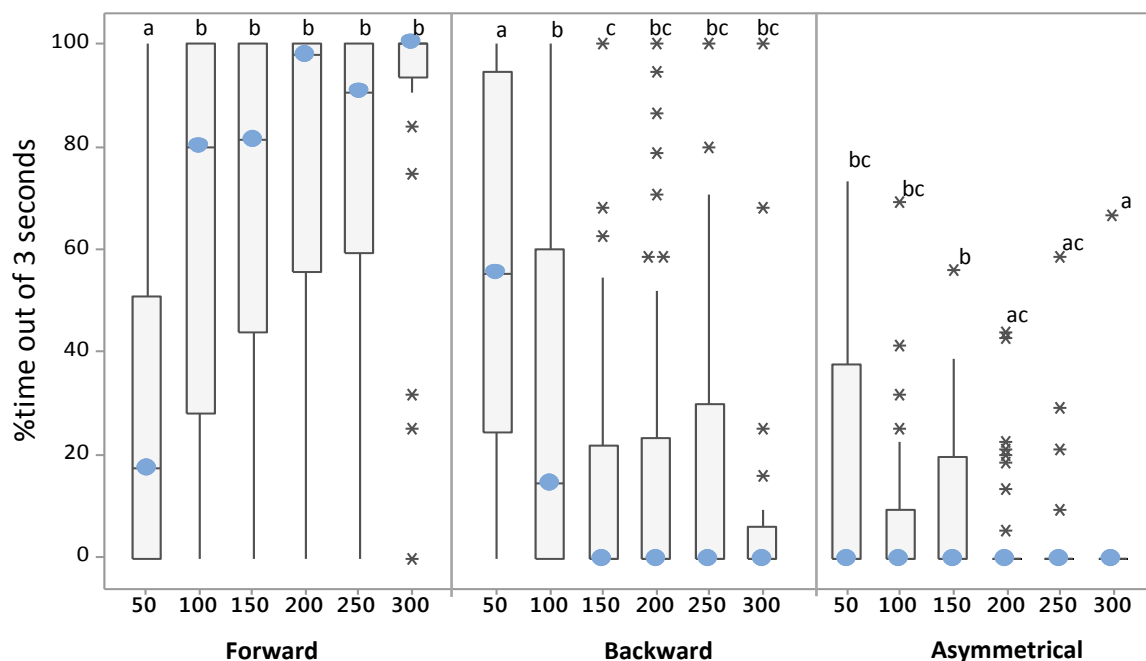


Figure 4.4. Effect of DistScreen, the estimated distance between the goat's head (tip of the nose) and the screen when the image appeared, on time spent with ears forward, backward and asymmetrical in 32 goats. DistScreen was divided into 6 categories, from 50 cm to 300 cm.  $P < 0.05$  when bars share no common letters. For each group, the median is indicated by the blue dot, the box represents the first and third quartiles, the upper whisker extends to the highest data value within the upper limit and the lower whisker extends to the lowest value within the lower limit. Values noted by an asterisk beyond the whiskers are outliers.

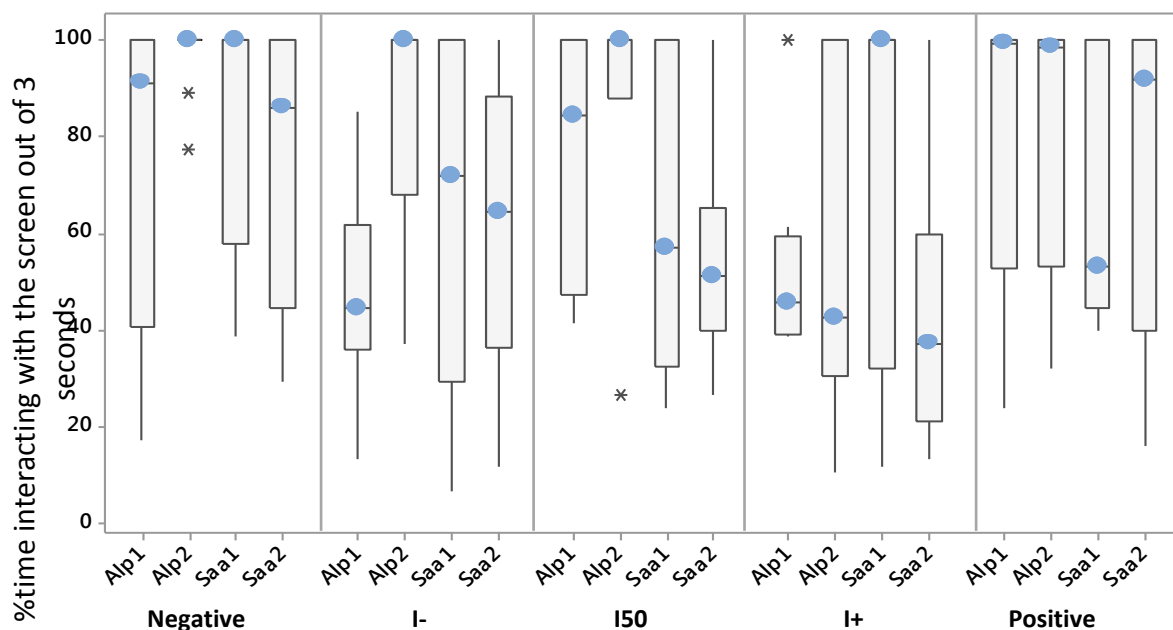


Figure 4.5 Effect of the interaction between the identity of the Photo Goat and the type of image shown. 'Negative': image taken while an icepack was applied to the goat udder. 'Positive': image taken while the goat was being groomed by a familiar experimenter. The three other types of images were of ambiguous facial expressions created using morphing software (25% positive (I-), 50% positive (I50), and 75% positive (I+)). For each group, the median is indicated by the blue dot, the box represents the first and third quartiles, the upper whisker extends to the highest data value within the upper limit and the lower whisker extends to the lowest value within the lower limit. Values noted by an asterisk beyond the whiskers are outliers.

Table 4.3 P-values for the effect of the interaction between the identity of the Photo Goat and the type of image shown on the percentage of time spent interacting with the screen. For readability reasons, the second half of the table has not been filled symmetrically. Significant differences ( $P < 0.05$ ) are indicated in bold text in Table (b).

		Neg.				I-				I50				I+				Pos.			
		Saa1	Saa2	Alp1	Alp2	Saa1	Saa2	Alp1	Alp2	Saa1	Saa2	Alp1	Alp2	Saa1	Saa2	Alp1	Alp2	Saa1	Saa2	Alp1	Alp2
Neg.	Saa1																				
	Saa2	0.55																			
	Alp1	0.17	0.09																		
	Alp2	0.56	0.37	0.13																	
I-	Saa1	0.82	0.84	0.17	0.51																
	Saa2	0.10	0.04	0.96	0.53	0.09															
	Alp1	<b>0.02</b>	<b>0.01</b>	0.08	<b>0.01</b>	<b>0.02</b>	0.25														
	Alp2	0.99	0.80	0.10	0.47	0.90	0.30	<b>0.003</b>													
I50	Saa1	0.87	0.56	0.11	0.57	0.74	0.21	<b>0.01</b>	0.90												
	Saa2	0.40	0.21	0.52	0.96	0.39	0.47	0.11	0.63	0.54											
	Alp1	0.87	0.56	0.11	0.57	0.74	0.21	<b>0.01</b>	0.90	0.47	0.54										
	Alp2	0.61	0.81	<b>0.02</b>	0.16	0.73	0.15	<b>0.001</b>	0.57	0.44	0.32	0.44									
I+	Saa1	0.06	0.16	<b>0.02</b>	0.09	0.18	<b>0.005</b>	<b>0.002</b>	0.32	0.09	<b>0.03</b>	0.09	0.57								
	Saa2	0.13	0.06	0.90	0.59	0.15	0.32	0.25	0.93	0.25	0.54	0.25	0.18	<b>0.004</b>							
	Alp1	<b>0.02</b>	<b>0.01</b>	0.10	<b>0.01</b>	<b>0.03</b>	0.31	0.95	<b>0.01</b>	<b>0.01</b>	0.12	<b>0.01</b>	<b>0.002</b>	<b>0.002</b>	0.26						
	Alp2	<b>0.02</b>	<b>0.01</b>	0.10	<b>0.01</b>	<b>0.03</b>	0.29	0.97	<b>0.009</b>	<b>0.01</b>	0.12	<b>0.01</b>	<b>0.002</b>	<b>0.001</b>	0.24	0.97					
Pos.	Saa1	<b>0.01</b>	<b>0.002</b>	0.91	0.50	<b>0.05</b>	0.94	0.20	0.29	0.12	0.41	0.12	0.12	<b>&lt;0.001</b>	0.97	0.22	0.21				
	Saa2	0.39	0.16	0.35	0.99	0.42	0.27	<b>0.05</b>	0.71	0.68	0.80	0.68	0.39	<b>0.01</b>	0.31	0.06	<b>0.05</b>	0.09			
	Alp1	0.17	0.09	0.98	0.12	0.17	0.95	0.12	0.10	0.11	0.52	0.11	<b>0.02</b>	<b>0.02</b>	0.90	0.12	0.10	0.90	0.34		
	Alp2	0.15	0.09	0.87	0.12	0.16	0.90	0.08	0.09	0.11	0.48	0.11	<b>0.02</b>	<b>0.02</b>	0.84	0.14	0.13	0.84	0.32	0.89	

### 4.3.2 Repeated exposure to the stimuli

The type of previous image seen (PrevIm) had a significant effect on time spent interacting with the screen ( $F_{5,86.8} = 11.54$ ,  $P < 0.001$ ) as well as on time spent in forward ( $F_{5,111.6} = 2.96$ ,  $P = 0.015$ ) and backward ( $F_{5,165.3} = 7.89$ ,  $P < 0.001$ ) ear postures (Figure 4.6). Post hoc analyses showed that this effect was due to the first session only, i.e. when there had been no previous image. Goats interacted with the screen for longer, spent more time with the ears forward and less time with the ears backward during the first Session than during any of the following sessions. PrevIm was not included in the final fitted model for the occurrence of asymmetrical ear postures.

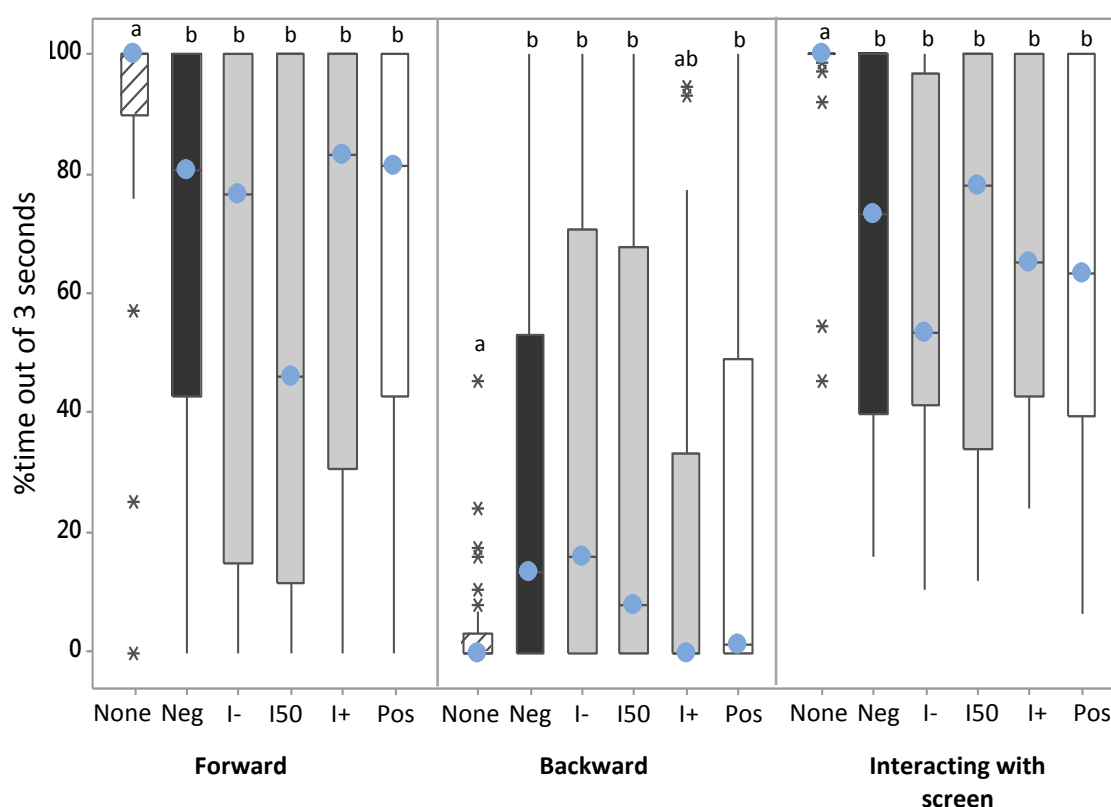


Figure 4.6. Effect of the type of previous image shown on the screen (PrevIm) on time spent with the ears forward and backward and on time spent interacting with the screen. None = no previous image (i.e. the first test session), 'Neg': the previous image was the face of a goat taken while an icepack was applied to the udder. 'Pos': the previous image was the face of a goat taken while the goat was being groomed by a familiar experimenter. The three other types of previous images were of ambiguous facial expressions created using morphing software (25% positive (I-), 50% positive (I50), and 75% positive (I+)).  $P < 0.05$  when the bars share no common letters. For each group, the median is indicated by the blue dot, the box represents the first and third quartiles, the upper whisker extends to the highest data value within the upper

limit and the lower whisker extends to the lowest value within the lower limit. Values noted by an asterisk beyond the whiskers are outliers.

Session 6, as a repeat of the first session, allowed a check of the validity of the goats' response to images of faces after five repeated exposures. Goats spent more time with the ears in forward ear postures when negative images were shown in both sessions (Session 1: Positive =  $82.2 \pm 29.9\%$ , Negative =  $97.3 \pm 6.9\%$ ,  $F_{1,22} = 9.62$ ,  $P=0.005$ ; Session 6: Positive =  $42.5 \pm 42.1\%$ , Negative =  $61.7 \pm 43.4\%$ ,  $F_{1,19} = 7.60$ ,  $P = 0.013$ ). For the other ear postures, results in Session 6 were in the same direction as those in Session 1; however, those differences were not statistically significant. Time spent with the ears forward during Session 1 was correlated with time spent with the ears forward during Session 6 ( $r_{p1-6} = 0.48$ ,  $P<0.006$ ). According to Martin and Bateson (2007) this indicates a "substantial relationship" between Sessions 1 and 6 where the same image was shown to a given individual goat (positive for some goats and negative for others). This relationship between Session 1 and 6 did not appear for other behavioural variables (ears backward:  $r_{p1-6} = 0.22$ ,  $P = 0.23$ ; ears asymmetrical:  $r_{p1-6} = 0.05$ ,  $P = 0.79$ ; time spent interacting with the screen:  $r_{p1-6} = 0.18$ ,  $P = 0.54$ ).

## 4.4 Discussion

### Differences in reactions to the different types of images

Our first hypothesis was that goats would show differences in their reactions to images of goats' faces taken in positive and negative situations, and that they would display behaviours indicating negative valence when looking at negative faces, and positive valence when looking at positive faces.

We found that goats displayed more ears forward when the image of a negative face was shown compared to a positive one. In sheep and goats, a higher percentage of

time spent with the ears forward has been observed in situations with a negative valence situations, such as when the animal is being pricked by an experimental device (Vögeli et al., 2014b) or when the animal is socially isolated (sheep: Reefmann et al., 2009c; goats: Briefer et al., 2015). However, a decrease in the percentage of time spent with the ears forward was observed after tail-docking and castration in lambs (Guesgen et al., 2016), suggesting that the association between ears forward cannot be generalised to all negatively valenced situations. In fact, forward ear postures have also been observed in situations where a high level of attention is required, i.e. eliciting high arousal (exposure to an unfamiliar test situation involving mild pain in sheep (Stubsjøen et al., 2009), or novel odour test in wild mice (Lecorps and Féron, 2015). Situations eliciting high arousal thus often coincide with a negative valence, but empirical observations have also identified forward ear postures in what could be considered positive situations, for instance, while the animals approached rapidly a bucket containing food pellets or when a familiar human entered the barn (personal observations). A higher percentage of time spent with the ears forward could then be associated with situations that lead to high arousal and/or increased attention, rather than to negative situations *per se*. Since most negative situations lead to an increase in attention to the environment (Carretié et al., 2001), this would explain the repeated occurrence of higher proportions of forward ear postures in negative situations.

Different situations can induce similar emotional states and facial expressions (including ear postures). For instance, social isolation (Briefer et al., 2015) and pain caused by castration and tail docking (Guesgen et al., 2016), both negative situations, have been associated with backward ear postures in small ruminants. It can thus be considered that here, goats perceived the valence of the situations as being positive or negative, rather than specificities of the situation, e.g. pleasurable handling or discomfort to the udder. To rule out alternative explanations would require repeating



these tests with images taken in two different positive and negative situations. Based on our results, images of faces taken during a negative situation seem to have elicited higher attention and arousal amongst the tested goats. This might indicate that images of faces taken during a negative situation were perceived as more negative stimuli by the goats, or at least elicited more attention than images of faces taken during a positive situation. From a behavioural ecology point of view, it is appropriate for prey animals such as goats to pay more attention to faces displaying negative emotions as they could signal the presence of potential threats such as predators, hunters or dangerous elements of the environment. The association of forward ear postures and increased attention in goats is further supported by the fact that the further a goat stood from the screen, the more time it spent with its ears forward. This could indicate that the animal was directing its attention towards the screen while keeping a safe distance, thus displaying higher alertness. It could also be hypothesised that goats standing further away had more difficulty to perceive the stimuli from a distance, and thus required higher attention. However the excellent visual acuity of goats (Blakeman and Friend, 1986) and the relatively short distance (3 m) makes this hypothesis unlikely. The lower proportion of ears forward observed when a positive image was shown would then indicate that goats were less attentive, and that the goats could have perceived images of faces taken during a positive situation as more positive or as less interesting.

In our study the percentage of time spent in backward ear postures was fairly low (20% on average), which represents an actual duration of less than 1 second. As such our results need to be treated with caution. Goats that were standing closer to the screen when the image appeared spent more time with their ears backward. In sheep, backward ear postures have been associated with novel and surprising situations (Boissy et al., 2011). If a goat was investigating the unlit screen closely, the suddenness

of the situation could have been perceived more strongly, which would explain this higher percentage of ears backward.

The percentages of time spent with asymmetrical postures were even lower ( $\leq 10\%$  on average), which is in agreement with observations made by Briefer et al. (2015). As pointed out by Guesgen et al (2016), although discrete ear postures were analysed, those postures were mutually exclusive. In other words, if the proportion of time spent with the ears forward decreased, the proportion of time spent in other ear postures increased. This could be another explanation for the higher percentage of time spent in asymmetrical ear postures that we observed when a positive image was shown. The three types of ear postures we recorded were indeed not independent and thus should be interpreted simultaneously. However, to identify how a situation was perceived, it is not the changes in ear postures, but rather the direction of the change (higher proportion of ears forward for instance) that is of interest.

Overall, these differences in ear postures indicated that goats paid more attention to images of conspecifics in a negative situation than to images of conspecifics in a positive situation. The fact that goats are able to identify faces of conspecifics in a negative situation, and so potentially a negative emotional state, could have welfare implications. From that perspective, it would be interesting to assess the impact of such images on the emotional state of the goat that is observing them. This, in the long term it might lead to a better understanding of the impact of seeing conspecifics in a negative emotional state and its implication from a welfare point of view.

### Potential use of images of faces in cognitive bias studies

If our results indicate that goats can discriminate between images of faces displaying different facial expressions of very opposite valence, it is still unclear which

facial features were indicative of the valence of the situation. Based on previous studies in sheep (Peirce et al., 2000; Tate et al., 2006), it is reasonable to assume that ear postures were important cues that the goats used to discriminate between images of faces. Variations in ear postures were also the most visible difference between the different types of images, at least to our human eyes. Additional studies using modified images hiding specific facial features (eyes, ears, mouth) would allow us to test this hypothesis (Wathan and McComb, 2014).

Our second hypothesis was that goats would show reactions to the morphed faces that were intermediate to the negative and positive images, and would reflect a gradual change in their response to the images, from the more negative to more positive image. However, the responses to morphed faces we observed were not necessarily intermediate for all behaviours (ears backwards and ears asymmetrical especially) and the variation in responses to morphed images was not gradual. This result is not encouraging regarding the use of images of faces for cognitive bias studies, because it does not agree with the two essential requirements for the use of cues for cognitive bias stated above. However it is worth noting that responses to the two extreme cues agreed with our hypotheses, and that difficulties arose with the morphed images. Morphed images have been used successfully as ambiguous stimuli in previous judgment bias studies in chickens (Salmato et al., 2011), but they consisted of silhouettes of birds and not complex stimuli such as faces. It is possible that the morphing did not take into account facial features that are of importance for face recognition or face based perception of emotions in goats, thus leading to images of that were less or not biologically meaningful to the test goats. This is why further work is thus needed to better understand how morphed images of faces are perceived by goats, and which facial features matter most for face-based perception of emotions.

## Methodological limitations

The identity of the Photo Goat affected the spontaneous reaction of goats to the images. This did not affect the general direction of the results, but it did affect more the magnitude of the responses. For instance goats tested with images of Photo Goat 'Saanen 2' displayed more forward ear postures overall, while still following the general response of a higher percentage of time spent with the ears forward when a negative face was shown. Dominance relationships, but also affinity between the tested and photographed individual, could have affected the goats' responses, which is why the addition of preliminary tests to determine inter-individuals relationships would have been a very useful addition to this study.

In this study, we presented the same image of a given Photo Goat in given situation to test goats. As a future refinement to this methodology, it would be important to understand whether a series of separate images of a given Photo Goat in a particular emotional state are perceived similarly within and between test animals. However, this study was a first step in investigating face-based emotion-recognition in goats, and allowed us to assess the effect of the type of image presented, with a satisfactory degree a generalisation (four different images were presented for each type of image). More studies would be needed, with experimental designs involving more images to determine how general these responses are.

We saw a strong effect of session on behaviour. Specifically, there was a difference between the first session and the other sessions in interest and attention. The higher interest for the image shown during the first session could be due to a novelty effect that quickly faded (Désiré et al., 2004). The presentation of the images was also not reinforced, and that could have lead to a drop in the goats' interest for the screen (Doyle et al., 2010b). However, even though the percentage of time spent

interacting with the screen dropped after the first session it stayed above 60% until the last session. In fact, even after five exposures to the stimuli, the goats still paid attention to the image.

Finally, the group of animals included in this study was as homogeneous as possible, especially in terms of age and previous experience. These factors may affect goats' responses. Further research would be thus be needed to clarify this point, since there is a possibility that this group differed from the general population for various reasons, including for example their past experience, the location of their home pen, the influence of one specific group member on the other animals, and difference in their relationships with humans. Choosing to include goats housed in separate pens, of varying ages and experiences could have lead to more generalizable results. However to avoid confounds between individual characteristics and, for instance, the type of image shown, we chose to study a homogeneous group.

## 4.5 Conclusion

Goats showed different reactions to images of faces photographed in different situations, indicating that they perceived the images as different. Goats also appeared more attentive towards negative images than towards positive or morphed images, which could indicate that negative images were, in fact, perceived to be more negative. Responses to morphed images were not necessarily intermediate to responses to negative and positive images and not gradual either, suggesting that using images of faces in cognitive bias tests may be inappropriate. Further study of the perception of morphed faces is needed. In addition, future research should take into account the fact that the goats appeared to be sensitive to the novelty of the stimulus and the identity of the individual in the photo.

## Chapter 5: Reactions of goats towards facial displays of emotions vary depending on dominance relationships

## Abstract

The existence of a rich repertoire of facial expressions in animals has been demonstrated and face-based emotion recognition is central to social behaviour, especially in species with complex social structures such as ungulates. In this study we investigated whether dairy goats ( $n=32$ ) displayed different responses to images of faces of familiar conspecifics taken in six situations that were all represented by a unique combination of valence and arousal (rumination, grooming, anticipation of food, unattainable food, ice pack applied to the udder, novel object). To that end, faces of four goats (Photo Goats) were photographed in these six situations. Prior to the presentation of images the dominance status of each test goat was determined through observations of competitive interactions at the feed face. The human-animal relationship was also assessed in an approach test. Each goat was then exposed for 3 seconds to each type of image with each test goat viewing images taken from a single Photo Goat. Presentation order was balanced across animals. Ear postures, rapid head movements (RHM) and time spent looking at the screen were recorded during presentation of the images. Scores representing response to handling in the experimental set-up were also recorded before each image presentation. Results were analysed using REML with subject identity as a random effect, so as to account for repeated measurements. Dominance activity within the herd and human-animal relationship only weakly affected responses ( $P<0.10$ ); however goats that had poor handling scores spent more time with their ears forward ( $F_{4,172.1} = 4.32$ ,  $P = 0.002$ ) indicating a higher attention towards the screen. Valence and arousal levels affected goats' behaviours independently. RHM increased with arousal level in the image ( $F_{1,179} = 6.49$ ,  $P = 0.012$ ) while time spent in backward ear postures was higher when the images were from positive situation ( $F_{2,73.8} = 4.9$ ,  $P = 0.010$ ). Time spent with the ears 'asymmetrical left' posture varied depending on the interaction between arousal level and dominance status of the Photo Goat ( $F_{1,137.6} = 3.59$ ,  $P = 0.024$ ). The interaction between valence and the dominance status of the Photo Goat influenced time spent with the ears pointed forward ( $F_{2,108.7} = 3.92$ ,  $P = 0.023$ ). Goats were more reactive when faced with images from subordinate Photo Goats. The influence of the dominance relationship highlights the importance of the interplay between social relationships and the expression and perception of emotions.

## 5.1 Introduction

The importance of faces in social interactions has been demonstrated in mammals, from non-human primates to ungulates. Faces convey information about identity, sex, age, gaze direction and emotional states to others (Leopold and Rhodes, 2010). Facial expressions in animals have recently received more attention, especially in non-primate species with less developed oro-facial musculature and where facial expressions might not be as easily apparent to human observers (Leopold and Rhodes, 2010). One essential tool to analyse facial expressions in humans is the Facial-Action Coding Systems or FACS (Ekman and Friesen, 1978). FACS are based on the anatomy of the face and on facial muscle movements. Facial expressions are split into Actions Units (AU), with each AU corresponding to the contraction of one specific muscle. FACS allow facial expressions to be described in a standardised way based on AU combinations, and allow us to identify similarities across emotional situations, cultures or even species. The FACS methodology has indeed been applied successfully to dogs (Waller et al., 2013), cats (Caeiro et al., 2013a) and horses (Wathan et al., 2015) and to several primate species (Vick et al., 2007; Parr et al., 2010). This work has demonstrated the existence of a rich repertoire of facial expressions in animals, with fascinating similarities to human facial expressions (Burrows, 2008). These facial expressions constitute both an emotional response and an element of social communication (Shariff and Tracy, 2011). Being able to recognise expressions of emotions in other individuals appears to be central to the social life of individuals, especially in species with complex social structures such as ungulates. Intra- but also inter-specific perception of facial expressions has been the focus of recent studies too, especially in horses. Horses are able to distinguish between expressions of positive and negative emotional states both in conspecifics and in humans (Wathan et al., 2016; Scheider et al., 2016; Smith et al., 2016).



In the previous chapter (Chapter 4), we concluded that goats reacted differently to images of faces of conspecifics experiencing situations of positive (grooming) or negative valence (ice block applied to the udder), indicating that this aspect of the facial display of emotions was perceived by the goats. However, since the positive situation also coincided with low arousal and the negative situation with high arousal, valence and arousal levels could be confounded in the images. This issue has been highlighted in previous research, where effects attributed to valence could have been the result of other aspects of the tests, and especially variation in arousal (Vögeli et al., 2014b). In the present study we sought to refine our analysis of goats' perception of emotions by being able to identify variations in reactions linked independently with valence or arousal. Images of faces of goats were obtained from six situations that elicited emotional states of varying valence and arousal. We predicted that goats would be more attentive towards images of faces taken during situations most likely to elicit a negative emotional state than towards images of faces taken in positive situations. This hypothesis was based on the established attention bias for negative stimuli in mammals, i.e. negative stimuli elicit a stronger attention and faster responses (Ito et al., 1998). Results from the three previous studies of this project (Chapter 2, Chapter 3, Chapter 4) also supported this hypothesis.

In previous chapters, the identity of the photographed animal influenced the animals' reactions to images of faces (Chapters 2 and 4). We thus wanted to test whether the dominance relationship between the test goat and the Photo Goat influenced the reactions of the test goat to different types of images. In social learning experiments involving foraging tasks in chickens, high-ranking individuals make better demonstrators than low-ranking ones (Nicol and Pope, 1999), potentially due to the adaptive value of paying attention to behaviours of dominant animals. Sheep have also been shown to be more attentive towards dominant conspecifics in foraging situations

(Hewitson et al., 2007). We thus hypothesised that test goats would be more reactive and more attentive towards faces of dominant Photo Goats.

We were also interested in examining the effect of individual characteristics, such as the human-animal relationship, on goats' reactions to images of faces. In an experimental set-up involving individual testing and therefore a high level of handling immediately before the test period, the human-animal relationship could indeed influence greatly the emotional state of the test goat at the time of exposure to the image. A goat stressed by handling immediately prior to the test could, for instance, be less attentive towards the test situation (Doyle et al., 2014b). If handling is stressful for some goats, it could potentially elicit cognitive biases, such as an attention bias towards negative images, i.e. stressed animals would pay more attention to negative images (Lee et al., 2016).

Finally, based on the results described in Chapter 4, we adjusted the experimental set-up to improve it and, notably, tried to minimise the impact of the novelty of the stimuli on goats' reactions. We also adopted a more fine-scale behavioural measure of ear postures, which would allow detection of more subtle variations in the reactions to images of faces. Asymmetrical ear postures, for instance, can be indicative of brain asymmetries in emotion perception (Basile et al., 2009). The right brain hemisphere bias for the processing of novel and threatening stimuli is well established in mammals (Rogers, 2002; Austin and Rogers, 2014). Hence, discriminating between two types of asymmetrical ear postures can be critical in identifying differences in reactions to different images of faces.

## 5.2 Methods

### 5.2.1 Ethical note

All experimental procedures were approved by the Animal Welfare Advisory Board of the research unit (INRA) and complied with the GRICE (Groupe de réflexion interprofessionnel sur les comités d'éthique appliquée à l'expérimentation animale or Interprofessional reflexion group on ethics committees applied to animal experimentation) recommendations. Animals were weighed weekly before, during and after the study, and were subjected to regular veterinary examinations as part of the farm schedule. No animals had to be removed from the study due to illness or injury.

### 5.2.2 Animals

This study took place at the INRA experimental farm of Thiverval-Grignon (France), during April and May 2016. Thirty-two primiparous lactating Saanen ( $n=16$ ) and Alpine ( $n=16$ ) goats of approximately 18 months of age were included in this experiment (those goats were different from the goats tested in Chapter 4). Two Saanen and two Alpine goats were selected to be filmed to produce images of faces used in testing, and are thereafter referred to as Photo Goats. All goats were housed in the same straw-bedded pen and were familiar with each other, having been reared together since separation from their dam after birth. Goats were milked twice a day, in the morning and in the afternoon. They were fed a standard ration once a day (mixed ration of hay, soy cake, barley and dehydrated alfalfa) and had unlimited access to water.

Since all 32 goats could not be tested in a day, they were split in two groups of 16 (Group 1 and 2), balanced for breed, human-animal relationship and the dominance relationship between the test goat and Photo Goat (see below for details of the

assessment of the latter two traits). Both groups were housed together in the same pen prior to, and during, the study (1.25 m<sup>2</sup> per goat).

### 5.2.3 Characterising the test goats

#### 5.2.3.1 Interactions at the feed face

Observations at the feed face were based on methods previously used in dairy cows (Rioja-Lang et al., 2012). The 32 goats were observed six times across eight days immediately after feed distribution, at peak feeding time (twice after morning feeding and four times after the afternoon sweep-in of leftover food). Each observation lasted 50 min. During observations, feed was only distributed in front of half of the feed faces. This limited access was necessary to increase the amount of competition. All interactions between any pair of goats competing for access to the feed face were recorded directly by one observer. Animals were also filmed during the observation period and any interactions missed from the live observations were obtained from the videos. If the interaction consisted of a goat coming into physical contact with another and which resulted in the feeding goat withdrawing from the feed face (i.e. displacement), the first individual was then recorded as successful. If the feeding goat repelled the aggressor and did not withdraw from the feed face, then the feeding goat was recorded as successful. A goat was considered dominant to another goat after being successful in at least two interactions with a given animal. If two animals had been successful in the same number of interactions with each other, their dominance relationship was considered unknown.

#### 5.2.3.2 Human approach test (HAT)

Approach tests are often used to assess the human-animal relationship on-farm and are suitable for goats given their curious temperament (Battini et al., 2016).

The human approach test (HAT) took place in the main building so that auditory and olfactory contact with other goats was maintained. The 32 test goats were brought to the milking parlour waiting pen, which was a familiar environment. A buffer pen (2 x 1.5m) separated that waiting pen from the actual test pen, which was the raceway (7.5 x 1.5m) used twice daily by goats to access the milking parlour. One side of this raceway was formed by the barred fence of the home pens, allowing visual contact with conspecifics. In the test pen, lines marked on the floor delimited 50-cm wide strips numbered from 1 to 15, with strip 1 the closest to the entrance (0-0.5m) and strip 15 (7-7.5m) the furthest. A stationary unfamiliar human stood in the strip furthest away from the entrance (strip 15, 7-7.5m) (Figure 5.1).

Each goat was tested once and Alpine and Saanen goats were tested alternately. The test started once the four legs of the goats were inside the test-pen. From that moment the goat had 180 seconds to touch the experimenter with its nose. If the goat touched the experimenter, then the experimenter tried to touch the goat reciprocally by calmly placing a hand on its head. The goat was then returned to its home pen. If the goat had not touched the experimenter after 180 s, it was returned to its home pen.

Latency to touch the experimenter in seconds was recorded as well as acceptance of touch (yes or no). The position of the goat's two forelegs in the test pen was recorded by scan sampling every 5 s (36 scans in total), using the number of the strip in which the goat was standing with its foremost leg. These variables were then used to rank the goats from most to least confident in their approach of the human. For the goats that touched the experimenter ranking was only based on the latency to touch the experimenter, with the more confident goats having shorter latencies. For the nine goats that did not touch the experimenter during the 180 s, an additional score was calculated, based on their relative position to the experimenter during the test. To that end, the strip numbers (1 to 14) recording the position of the goat in the test pen at each scan sample were summed for the 36 scans. A higher sum meant the goat had

approached closer to the experimenter. For instance, a goat that did not touch the experimenter, walked up to strip 7 but spent most of the 180s in strip 3 (3-3.5 m from the entrance), had a higher sum than a goat walked up to strip 3 (1-1.5 m from the entrance) and that spent most of the 180s also in strip 3. This ranking was then used to balance the experimental groups for human-animal relationship.

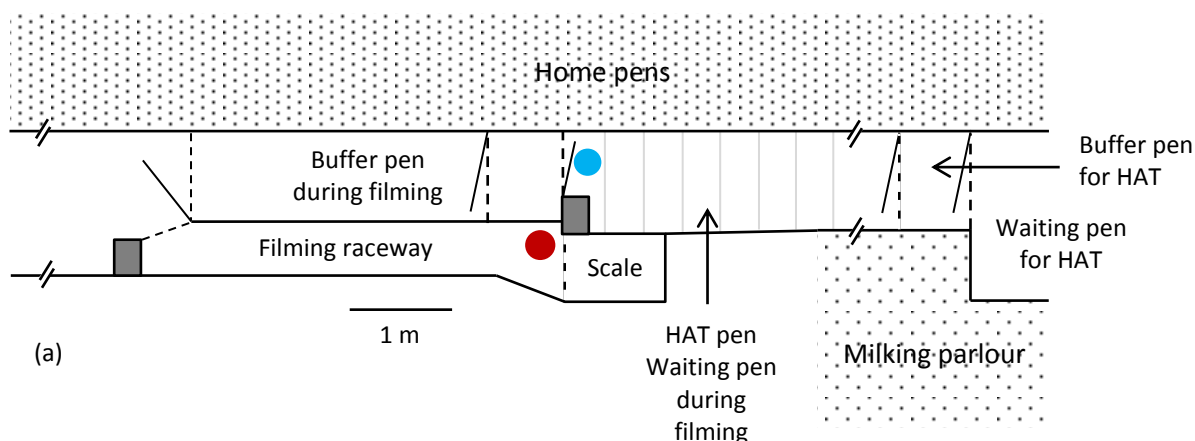


Figure 5.1. Set-up used for both the human-approach test (HAT) and for taking pictures of faces. The blue dot represents the location of the stationary unfamiliar experimenter during the HAT and the red dot the location of the filming experimenter in the raceway during the six situations used to induce emotional states of varying valence and arousal

#### 5.2.4 Choice and allocation of Photo Goats

The choice of Photo Goats was based on the hierarchical relationships between animals within one breed, since each test goat looked only at images from a Photo Goat of its own breed. A success index was calculated based on the feed face observations (see below Section 5.2.8.1 for details) and the Photo Goats were the two individuals with the highest success index within their breed. The dominance relationship between each Photo Goat and all the other goats from the same breed was also known from the feed faces observation. In cases where no spontaneous interactions had been observed at the feed face for any pairing between a Photo Goat and a test goat of the same breed, the two goats were placed together in a small familiar pen where only one feed face was open and presented with concentrate. Competitive interactions were recorded as described in Section 2.2.1 and the dominant goat was identified as far as possible, since

for some pairs the same number of displacements had been recorded for both goats. Each Photo Goat was dominant to at least nine of the fifteen goats from its own breed.

Eight test goats were allocated to each Photo Goat based on their dominance relationship. A test goat was only allocated to a Photo Goat if the dominance status between the two individuals was clearly established. So, if the same number of successful displacements had been recorded for both goats, then the test goat was not allocated to this Photo Goat. Moreover, even though the Photo Goats had the highest Success Index and were dominant to the majority of individuals within their breed, due to the non-linearity of hierarchy in the herd, some test goats were found to be dominant to both Photo Goats of their own breed. This is why each Photo Goat was dominant over six test goats and subordinate to two test goats. This also allowed us to investigate the impact of the dominance relationship between the test goat and the Photo Goat (RelPG) on the reactions of test goats to images of faces. However this experimental design was unbalanced, as only the Photo Goats were subordinate to only 8 test goats while dominant to 24 test goats. This constraint in experimental design was taken into account into the interpretation of our results.

### 5.2.5 Taking pictures

Photo Goats were filmed in six situations that were considered to induce different emotional states: rumination, grooming, anticipation of food, presentation of unattainable food, application of an icepack to the udder and presentation of a novel object. The determination of the emotional states elicited by the situations is detailed in Section 5.2.6. The situations were filmed consecutively following this same order. This order was selected as to avoid confounding effects between two consecutive situations. This is why we started with the more positive situations (grooming, food), to prevent any avoidance behaviour that could arise in the goats after experiencing a negative situation in a given location. This also allowed us to use the contrast between

the pleasant situations followed by unpleasant ones in the same location, as it has been shown that positive-negative contrasts are perceived as negative by sheep (Reefmann et al., 2009a; Greiveldinger et al., 2011). For the food-based situations, it was also necessary to start with the presentation of food, so that goats expected the food to be available during the following presentation of unattainable feed. For all situations except rumination, filming took place in the raceway leading to the weigh crate and located in the main building (Figure 5.1). The filming experimenter was not hidden but was located in the same spot for all situations. The very high habituation of the goats to human presence meant that they did not pay attention to the experimenter, which was considered only a part of the set-up. This set-up was well known by the animals as they are weighed weekly, and so the set-up was not considered stressful.

The face of each Photo Goat was filmed with a HD camera (HDR-XR155, Sony, Japan). After review of the video clips collected, the most representative facial expression observed during the situation was identified. This consisted of a facial expression consistently presented by all individuals filmed during the situation. The facial expression also had to occur during the same time-window for all individuals (e.g., within 50 sec of the beginning of the isolation). The selection of this representative facial expression was also based on the general behaviour expressed by the animal, as detailed below for each situation. We chose to use a frontal view of the faces, as it was the best way to show the maximum of facial features at the same time to the goat observing the images (e.g. both ears and eyes visible at the same time). A frontal view of the animal face displaying the most representative facial expression observed during the situation was thus extracted from the video clips using Pinnacle Studio 17 (Pinnacle Systems, 2013). Faces were then digitally cut and placed against a neutral beige background using Adobe Photoshop CC (Adobe Systems, 2014).



#### 5.2.5.1 Rumination

Goats were filmed in their home-pen while lying down and ruminating. The ears were hanging horizontally, with the auricles facing forward (Figure 5.2). These images are referred to as 'Rumination'.

#### 5.2.5.2 Grooming

Goats were habituated to grooming in the home pen the day before filming took place. At the end of the habituation, the Photo Goats did not move away and after grooming they repeatedly sought attention from the experimenter. These observations supported the idea that grooming was pleasurable. Then one Photo Goat was brought to the raceway while the other Photo Goats waited in the buffer pen, allowing visual contact with conspecifics (Figure 5.1). An experimenter standing behind the goat groomed the animal for two minutes. Grooming consisted of gentle scratching and massaging of the sides of the neck, which has been shown to be appreciated by sheep and cattle (Schmied et al., 2008; Reefmann et al., 2009c). Goats had their ears lowered and turned down during almost the entire grooming session, and pictures of the animal displaying this ear posture were extracted from the videos (Figure 5.2) and are referred to as 'Grooming' from this point onwards.

#### 5.2.5.3 Anticipation of food

A bowl containing pellets of concentrate feed was placed at the end of the raceway and visible from the entrance. Each Photo Goat was given a first run through the raceway and allowed a mouthful of concentrate before being filmed during a second run, as it voluntarily approached the highly appetent food and ate it. Pictures were extracted when the goat had both the tip of the ears and the auricle pointing forward towards the food (Figure 5.2) and these pictures are hereafter referred to as 'Food'.

#### 5.2.5.4 Presentation of unattainable food

This situation was filmed in a third consecutive run, immediately after the Photo Goat had eaten from the bowl, which was now closed with a transparent lid. The Photo Goat was brought back to the entrance of the raceway, as described above and was left to interact with the closed bowl for 5 minutes. Each Photo Goats first interacted actively with it, nibbling at it, pushing or kicking it with the forelegs. After intervals ranging from 30 s to 2 min, the Photo Goat withdrew and tried to exit the raceway. Pictures were extracted from the videos at the time of withdrawal from the bowl, when goats were standing with the head raised and the tip of the ears pointing backward (Figure 5.2). These images are thereafter named 'Resignation'.

#### 5.2.5.5 Ice pack on the udder

Goats were individually in the weigh crate at the end of the raceway. An experimenter applied an ice pack to the udder until a negative reaction (e.g. sharp head movements, stamping, trying to escape the weigh crate) was observed. Pictures from the first reaction of the goat were extracted from the films, when the animal raised its head, with the tip of the ears pointing backward and the auricles turned downwards (Figure 5.2). These images are named 'Ice'.

#### 5.2.5.6 Novel Object

A novel object (red ball approximately 20 cm in diameter) was placed at the end of the raceway. Each goat was left in the raceway to interact with the novel object for 2 min. When each Photo Goat touched the ball for the first time, it displayed the same asymmetrical ear postures, with the right auricle turned forward and the left auricle turned backward. Pictures corresponding to that first interaction were extracted from the videos (Figure 5.2) and named 'Novel Object'.

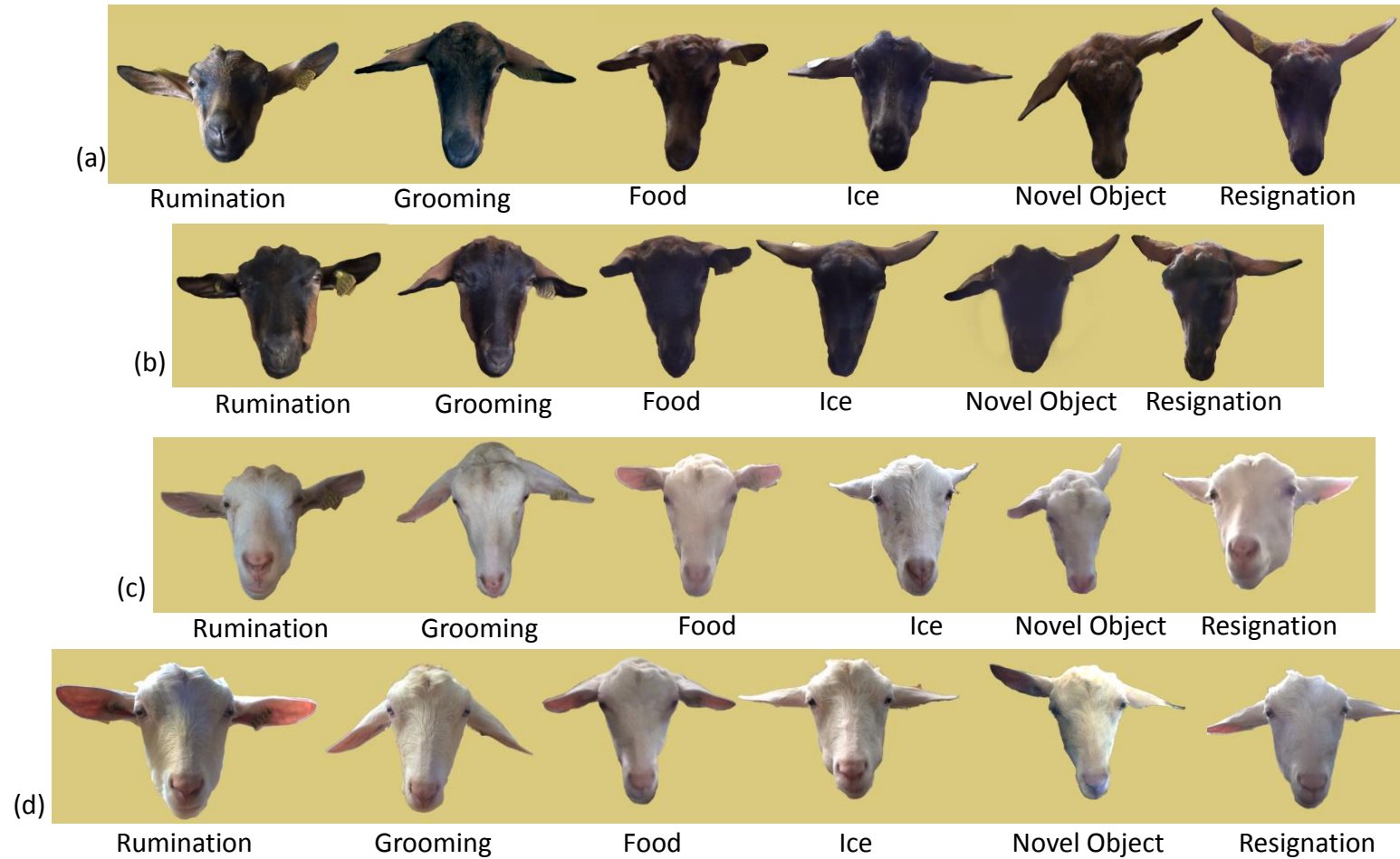


Figure 5.2. **The six types of images of faces obtained from four different goats: (a) Photo Goat Alpine 1 (b) Photo Goat Alpine 2, (c) Photo Goat Saanen 1, (d) Photo Goat Saanen 2.** ‘Rumination’ images were taken while the goat was lying ruminating in the home pen, ‘Grooming’ images were taken while the goat was being groomed by a familiar experimenter, ‘Food’ images were taken while approaching a highly appetent food reward, ‘Ice’ images were taken when an icepack was applied to the udder, ‘Novel Object’ images were taken during the first contact with a red ball and ‘Resignation’ images were taken once the goat had stopped interacting with a closed box containing food and withdrew from it.

### 5.2.6 Determining valence and arousal levels for each situation

For each situation, valence and arousal levels were inferred from the behaviour of the Photo Goat behaviour in conjunction with the functional framework of emotions proposed by Mendl et al. (2010). Stimuli enhancing fitness or rewarding stimuli are thought to elicit positive emotional states, while fitness-threatening or punishing stimuli (predator, pain, stress) elicit negative emotional states. Similarly to the method presented by Briefer et al (2015), valence levels were arbitrarily attributed as either negative (-1), neutral (0) or positive (+1), while arousal levels were split between low (1) and high (2) (Table 5.1).

Rumination was considered to be a relaxed state of neutral valence and low arousal, similar to the one described in horses by Wathan et al. (2016) . Grooming has been shown to induce a positive judgment bias in goats (Baciadonna et al., 2016). Moreover, goats repeatedly sought attention from the experimenter after the first grooming sessions in their home pen, and their relaxed posture during grooming (ears hanging loose, lying down, eyes closing) led us to consider that Grooming induced a situation of positive valence and low arousal. During Food, goats approached the bowl very rapidly and short-term anticipation of food has been shown to induce positive emotional states (Boissy et al., 2007). Accessing a food reward is a fitness enhancing situation, and Food was thus assumed to induce a positive emotional state with a high level of arousal.

On the other hand, unattainable food represents a situation threatening fitness and this situation was thus considered to have induced a negative emotional state (Doyle et al., 2011). Frustration from thwarting the motivation to obtain food firstly resulted in a negative emotional state of high arousal (kicking the bowl, vocalisations, stamping). However, when the pictures for 'Resignation' were taken, the Photo Goat was standing still and at a distance from the closed bowl. The withdrawal from the stimulus suggests that goats were thus in a negative state of low arousal akin to resignation or sadness in humans (Ellsworth and Smith, 1988). The attempts

made by the Photo Goat to avoid the ice pack and escape from the weigh crate indicated discomfort. Ice was thus considered to have induced a negative state of high arousal.

All Photo Goats showed increased activity when presented with the novel object (head movements, ear movements, locomotion), and Novel Object was thus assumed to be a situation of high arousal (Briefer et al., 2015). Goats are generally considered to be curious animals that investigate their environment (Miranda-de la Lama and Mattiello, 2010). However goats also display individual differences in their exploratory behaviour, and investigating a novel object could be more or less positive depending on the position of the animal on the shy/bold personality dimension (Nawroth et al., 2017). All Photo Goats approached the novel object voluntarily, but at varying speeds, which could indicate different subjective experiences of the situation. A very curious or bold goat for instance would approach the object very quickly to investigate it, while a shyer animal would stay at a distance for a longer period of time and approach it more cautiously. Consequently, an overall valence of 0 was attributed to Novel Object.

Table 5.1. Valence and arousal levels determined for each situation where photos of individual goats were taken.

<b>Situation<sup>1</sup></b>	<b>Valence</b>	<b>Arousal</b>
<b>Rumination</b>	0	1
<b>Grooming</b>	+1	1
<b>Food</b>	+1	2
<b>Resignation</b>	-1	1
<b>Ice pack</b>	-1	2
<b>Novel object</b>	0	2

<sup>1</sup> Rumination: goat lying and ruminating in the home pen; Grooming: pleasurable grooming by a human handler; Food: presentation of a highly appetent food in an open bowl; Resignation: presentation of unattainable food in a closed bowl, images taken after withdrawal from the bowl; Ice pack: ice pack applied to the udder; Novel object: presentation of a novel object in a familiar raceway.

## 5.2.7 Spontaneous reactions to images of faces

### 5.2.7.1 Test pens

Tests took place in a covered area located approximately 40 m outside the main building (Figure 5.3). A waiting pen was adjacent to the test pens but was separated by a wall of straw bales. A small buffer pen next to the waiting pen allowed the experimenter to isolate one goat at a time for testing. A two-way raceway with solid walls connected the waiting pen to the two test pens (video presentation pen and screen pen). The first raceway led from the buffer pen to the video presentation pen (1 x 0.6 m) preceding the screen pen. The video presentation pen was separated from the screen pen by a 70 cm high sliding-gate allowing a goat placed in the video presentation pen to see inside the screen pen. A computer screen was placed in the screen pen on the wall opposite the sliding-gate and at eye-level for a goat. The exit-gate from the screen pen was connected to the buffer pen through the second race-way. The test pens were filmed by two cameras, one above the screen pen and one behind the computer screen.

### 5.2.7.2 Habituation

Habituation took place across two days prior to the test sessions and was divided into five stages. The 16 goats from one group were brought on a leash to the waiting pen. We used a systematic desensitization procedure, i.e. an animal only moved onto the next stage once exposure to the previous stage no longer resulted in a stress response. Stages 1, 2 and 3 took place on the first habituation day. For the first stage, all gates were open in the raceways and test pens and goats were walked individually through the set-up by an experimenter moving slowly behind them, until they entered the video presentation pen smoothly. This took between one and five consecutive runs. For the second stage, both the goat and the experimenter were confined in the screen pen for ten seconds before the exit-gate to the raceway was opened. It took between one and three runs before all goats performed this calmly. During the third stage, goats were confined alone in the video presentation pen for five seconds, with the sliding-gate

closed and then had to enter the screen pen by themselves before exiting through the return raceway. This took one or two runs. The fourth and fifth stages took place on the second day of habituation. The fourth stage consisted of a repeat of the third stage, but goats were confined in the screen pen for ten seconds (for two runs). The fifth stage was a sham test session: an image of an inverted grey triangle on the same beige background that would then be used with images of faces was shown on the screen for a duration of 3s, as this was the exposure time that was then used during the test sessions (see Section 2.6.3 for details).

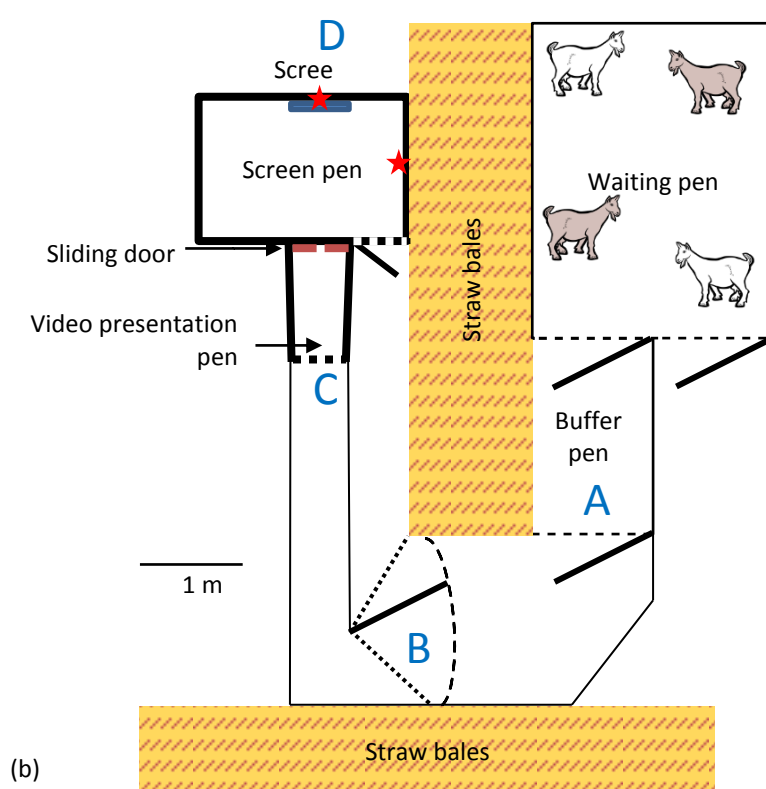


Figure 5.3. Set-up for the presentation of images of faces. Red stars indicate the positions of the camera. Thick black lines indicate opaque walls. Handling score H<sub>Sa</sub> was recorded from A to B and handling score H<sub>Sb</sub> from B to C. The hidden experimenter stood at position D and was responsible for directing the goats' attention towards the screen by moving colourful items above the screen.

### 5.2.7.3 Test sessions

The three days of testing immediately followed the habituation phase. All goats were exposed to the six types of images described, one per test session, with two test sessions per day. There was a total of 12 test sessions, six within each group. Within a group, order of testing

was balanced for breed, identity of the Photo Goat, relationship between the Photo Goat and the tested goat (dominant or subordinate) and human-animal relationship. Order of images presented to each goat was balanced across sessions and was different for each goat.

One hour after morning milking, 16 goats were brought on a leash to the waiting pen. Once the last goat entered the waiting pen, goats were given 15 minutes to settle down before the beginning of the tests (i.e. until some goats started lying down in the waiting pen). A goat was then brought to the video presentation pen and two different handling scores (HS) were recorded: HS<sub>a</sub> from the buffer pen (A) until gate B (Figure 5.3) and HS<sub>b</sub> from gate B until the goat was in the video presentation pen (C). HS<sub>a</sub> characterised the ease of separation from the group, while HS<sub>b</sub> characterised how easily the animal would enter a small confined space. HS were assessed on a 5-points scale, based on the current practices at the experimental farm, where 1 characterised the most docile animals (Table 5.2). Once in the video presentation pen, the goat was faced with the blank screen. Before showing an image on the screen, we wanted to be certain that the goat was directing its attention towards it, and so that it would react to the stimulus presented only. The image was thus only displayed once the goat was 'paying attention' to the screen, i.e. staring at it for at least one second. An experimenter hidden behind the screen wall (position D, Figure 5.3) directed the goat's attention towards the screen by moving colourful items above the screen through a small hole in the solid wall. The experimenter switched to a different item every ten seconds until the goat reached the 'paying attention' criterion, and the order of presentation of the items was the same for every goat. If more than three different items had been used unsuccessfully or if the goat climbed on the sliding-gate, the hidden experimenter started speaking to attract the goat's attention, using standardised sentences and sounds. As soon as the goat reached the 'paying attention' criterion, an image was displayed on the screen for 3 seconds. Once the image disappeared, the sliding-gate was opened and the goat could enter the screen pen. The goat was given ten seconds to interact with the screen, from the moment its four legs were inside the screen pen, before it was brought back to the waiting pen. All goats were tested successively for the first session of the



day, and then tested again following the same order for the second session after a break of 30 minutes. For a given goat, the two daily sessions were thus separated by approximately 80 minutes. Within a group, testing order was the same for all six sessions.

Table 5.2. Handling score scale used for handling scores HSa and HSb

Handling Score	Description
1	No vocal encouragement, no contact, only walking behind the goat
2	Vocal encouragement, no contact
3	Vocal encouragement, touch without pushing
4	Vocal encouragement, light push
5	Vocal encouragement, strong push

## 5.2.8 Data collection and analysis

### 5.2.8.1 Individual characteristics

A success index adapted from Mendl et al. (1992) was calculated for each animal using the interactions recorded at the feed face. The success index was indicative of the dominance activity for a given goat. It was preferred to the establishment of a dominance hierarchy due to the high number of pairs not observed interacting at the feed face and to the frequent reversals (both individuals from one pair won in two separate interactions)

$$\text{Success index} = \frac{\text{Total number of successes}}{\text{Tot. number successes} + \text{Tot. number losses}} * 100.$$

The human-animal relationship ranking used to balance the experimental groups was replaced by a new parameter for the analysis. Latency to touch the human handler was recorded during the HAT and the scan sampling of the goat position in the test pen provided a measure of the maximum distance from the entrance in the direction of the human in the test pen. However, the goats that did not touch the human handler were all given a maximum latency of 181 s (time limit set at 180 s), so including the latency only in our analyses would not have distinguished between these animals (Figure 5.4a). Similarly the goats that touched the handler all had a maximum distance from the entrance of the test pen of 7.5m (equivalent to

strip 15). These goats could thus not be distinguished based on this parameter only (Figure 5.4b), while for goats that never touched the human the maximum distance from the entrance ranged between 1.5 and 6.5m. A new parameter, HumApproach, was thus calculated based on these variables. For each goat, latency to touch the human handler (in seconds) was divided by the maximum distance by the goat from the entrance of the test arena (in meters). The calculation of HumApproach allowed both types of goats (that touched or did not the handler) to be placed on a common continuous scale, and to quantify their relative confidence to approach humans (Figure 5.4c).

$$\text{HumApproach} = \frac{\text{Lat. to touch Human (s)}}{\text{Maximum distance from the entrance (m)}}$$

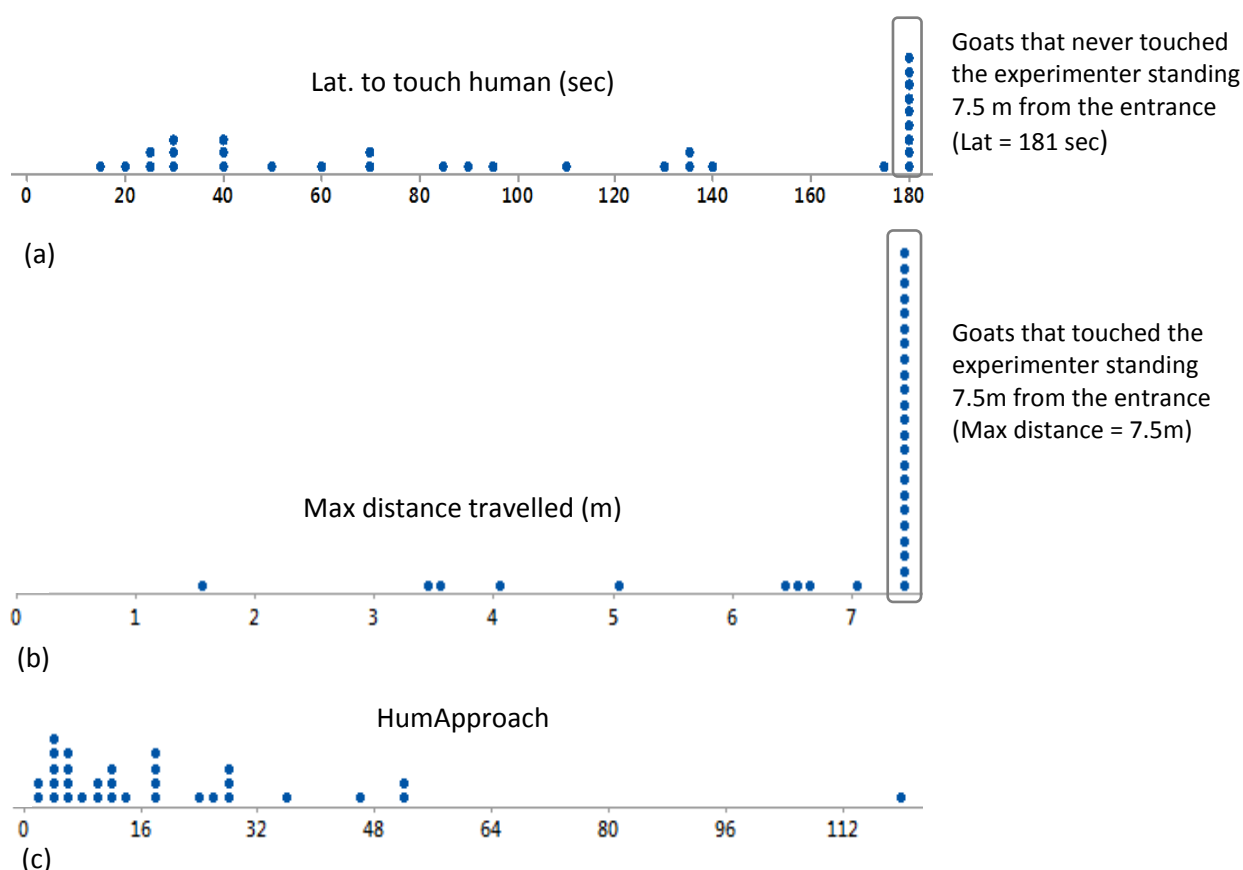


Figure 5.4. **Outcome variables from the human-approach test.** (a) **Latency to reach the experimenter (sec)**, 32 goats had 180 sec to touch a stationary human at the other extremity of the test pen. (b) **Maximum distance travelled in the test pen (m)**, the maximum distance was 7.5m for goats that touched the experimenter, but varied for goats that did not. (c) **HumApproach**, calculated parameter for each goat,  $\text{HumApproach} = \text{Latency to touch human} / \text{maximum distance travelled}$ . D places all goats on a common continuous scale. Each blue dot represents one goat.

### 5.2.8.2 Data collection and statistical analyses

Behaviours described in Table 5.3 were extracted and scored from the video recording for the 3 seconds interval when the image was displayed in each test session using The Observer 5.0 (Noldus Information Technology, Netherlands). The observer was blind to the type of image shown and the relationship between the Photo Goat and the test goat. Video playback speed was slowed down by a factor of 10 for behavioural observations, to allow for fine-scale recording of ear postures. All changes in ear postures were recorded.

The outcome variables were percentages of time spent in each ear posture described in Table 5.3 (F-raised, F-pointed), backward, AsymLeft and AsymRight), the percentage of time spent looking at the screen, the number of rapid head movements (RHM) and the number of approaches towards the screen in the screen pen (ApproachScreen). Time spent in each ear postures sums to the total duration of the observation (i.e. sum-one constraint). Outcome variables expressed as percentages of the total test duration were logit-transformed to conform to assumptions of normality and homogeneity. The total numbers of changes in ear postures and of rapid head movement as well as the predictor variables LatCrit, HumApproach and the success index were natural log transformed for the same reasons.

Analyses were conducted using GenStat 16<sup>th</sup> edition (VSN International Ltd., United Kingdom). Significance level was set at  $P = 0.05$  and data in the text are presented as means  $\pm$  standard deviation, unless stated otherwise. For continuous variables, data were analysed by linear mixed models (REML) with repeated measurements. A power model was used to account for correlations within subjects across time. Power models allow unevenly spaced time points to be taken into account (e.g. that Sessions 1 and 2 were on the same day and thus closer in time than Sessions 2 and 3), since the correlations between measurements decrease as time between measurements increases. Heterogeneity of the variance was allowed when it led to a smaller deviance (one-tailed test with a  $\chi^2$  distribution). ApproachScreen could not be logit

transformed to meet the required statistical assumption and was transformed to binomial data (0 = approached, 1 = did not approach) and analysed with a general linear mixed model (GLMM) with a binomial distribution and logit link function. Normality of residuals was checked graphically. Post-hoc pairwise comparisons were conducted using Fisher's least significant difference tests.

The different types of images were broken down into a valence (ValenceIm) and an arousal (ArousalIm) component (see 2.4 Determining Valence and Arousal for each situation). The combination of valence and arousal was unique for each type of image, thus the ValenceIm.ArousalIm interaction represented the type of image in our model. A similar approach was used to code the type of previous image shown (PrevIm) in ValencePrevIm and ArousalPrevIm, with their interaction representing the type of PrevIm. Separate mixed models were fitted by backward stepwise selection for each outcome variable (Table 5.4). The possible fixed effects fitted for each outcome variable included Breed, valence of the image (ValenceIm), arousal of the image (ArousalIm), the ValenceIm.ArousalIm interaction, identity of the Photo Goat (iPG), dominance relationship between Photo Goat and test goat (dominant or subordinate, abbreviated RelPG), ValencePrevIm, ArousalPrevIm and the ValencePrevIm.ArousalPrevIm interaction, the handling scores (HSa and HSb), LatCrit, HumApproach and the success index. Animal and Session were always included in the random effects as Animal nested within Session for the REML and Animal and Session for the GLMM. Session was coded from 1 to 12 to account for the two experimental groups: Sessions 1 to 6 corresponded to Group 1 and Sessions 7 to 12 to Group 2.

Table 5.3. Recorded behaviours and transformation applied to outcome variables in goats shown images of familiar conspecifics on a screen for 3 s.

Behaviour		Abbreviation	Description	Transformation	Type
Beginning of the test		---	Once the gate of the video presentation pen was locked behind the goat and the handler hidden	---	---
Latency to pay attention to the screen		LatCrit	Latency between the beginning of the test and the display of the image on the screen once the goat had reached the 'Paying attention' criterion.	Ln	Explanatory
Looking at Screen		Looking	Time spent looking at the screen with both eyes while the image is displayed on the screen	Logit	Outcome
Ear postures	Ears forward raised	F-raised	Tip of both ears pointing towards the front, at an approximately 30° or more from a perpendicular axis	Logit	Outcome
	Ears forward pointed	F-pointed	Tip of both ears pointing towards the front, at approximately 60° or more from a perpendicular axis	Logit	Outcome
	Ears backward	Back	Tip of both ears pointing towards the back,	Logit	Outcome
	Ears asymmetrical right	AsymRight	Tip of right ear pointing forward and tip of left ear pointing backward	Logit	Outcome
	Ears asymmetrical left	AsymLeft	Tip of left ear pointing forward and tip of right ear pointing backward	Logit	Outcome
	Changes in ear postures	Changes EP	Total number of changes in ear postures during a test session	Ln	Outcome
Rapid Head Movements		RHM	Total number of rapid (i.e. < 1s) head movements in any direction (adapted from Briefer et al., 2015)	Ln	Outcome
Approached screen		ApproachScreen	Once released from the video presentation pen, walked towards the screen while staring at it, bringing the head closer to the screen	Binomial	Outcome

Table 5.4. Recorded behaviours and transformation applied to outcome variables in goats shown images of familiar conspecifics on a screen for 3 s.

<b>Outcome variable<sup>1</sup></b>	<b>Final fitted model</b>
<b>F-raised</b>	Breed + ArousalIm + iPG + RelPG + ArousalIm.iPG + HSa + HumApproach
<b>F-pointed</b>	Breed + ValenceIm + ArousalIm + iPG + RelPG + ArousalIm.iPG + Valence.RelPG + ArousalPrevIm + ValencePrevIm + ArousalPrevIm.ValencePrevIm + HSa + HumApproach
<b>Backward</b>	Breed + ValenceIm + ArousalIm + iPG + RelPG + Arousal.RelPG + HSa
<b>AsymRight</b>	Breed + ValenceIm + ArousalIm + iPG + RelPG + ValenceIm.iPG + ArousalIm.iPG + Arousal.RelPG + ArousalPrevIm + ValencePrevIm + ArousalPrevIm.ValencePrevIm
<b>AsymLeft</b>	Breed + ValenceIm + ArousalIm + iPG + RelPG + ValenceIm.iPG + Arousal.RelPG + ArousalPrevIm + ValencePrevIm + HSa + HSb + LatCrit + HumApproach + SuccessIndex
<b>Looking</b>	Breed + ValenceIm + iPG + ValenceIm.iPG + HSa + HumApproach + SuccessIndex
<b>Changes EP</b>	Breed + ValenceIm + ArousalIm + iPG + RelPG + Valence.ArousalIm + ArousalIm.iPG + ValencePrevIm + HSa + HumApproach
<b>RHM</b>	ArousalIm + RelPG + ArousalPrevIm + HSa + HSb
<b>Approached</b>	Breed + ArousalIm + iPG + RelPG + ArousalIm.iPG + ArousalPrevIm + ValencePrevIm + ArousalPrevIm.ValencePrevIm + HSb + LatCrit + SuccessIndex

<sup>1</sup>F-raised: time spent with the ears in forward raised posture out of 3 s, F-pointed: time spent with the ears forward pointed, Backward: time spent with the ears backward, AsymRight: time spent with the right ear forward and the left ear backward, AsymLeft: time spent with the left ear forward and the right ear backward, Looking: time spent looking at the screen, Changes EP: total number of ear postures changes recorded, RHM: total number of rapid head movements, Approached: goat approaches of the screen when leaving the video presentation pen (binomial).

## 5.3 Results

### 5.3.1 Spontaneous reactions to images

The type of image, represented by the ArousalIm.ValenceIm interaction, had no direct effect on any of the behaviours. However, the arousal and valence characteristics of the images affected the goats' spontaneous reactions independently.

ArousalIm had a direct effect on RHM only. Goats displayed more RHM when the image shown was from a situation of higher arousal (ArousalIm = 1:  $2.4 \pm 0.9$  RHM; ArousalIm = 2:  $2.9 \pm 1.1$ , RHM;  $F_{1,179} = 6.49$ ,  $P = 0.012$ ). The interaction between ArousalIm and identity of the Photo Goat had an effect on time spent in the F-raised ear posture ( $F_{3,152.3} = 3.45$ ,  $P = 0.018$ ). Time spent with the ears in F-raised posture did not differ between images of low and high arousal for test goats observing images of Photo Goats Saanen 1, Saanen 2 and Alpine 2 (Saanen 1:  $F_{3,152.3} = 3.45$ ,  $P = 0.799$ , Saanen 2:  $F_{3,152.3} = 3.45$ ,  $P = 0.385$ , Figure 5). On the other hand, goats exposed to images of Alpine 1 spent longer with their ears in F-raised posture when the image was from a situation of low arousal (ArousalIm = 1) compared to images from situations of high arousal (ArousalIm = 2) ( $F_{3,152.3} = 3.45$ ,  $P = 0.007$ , Figure 5.5). The interaction between ArousalIm and the dominance relationship between the Photo Goat and the test animal (RelPG) affected the time spent with the ears in AsymLeft (left ear forward, right ear backward). When the Photo Goat was their subordinate, test goats spent longer with their ears in AsymLeft when exposed to an image taken in a situation of high arousal than in a situation of low arousal ( $F_{1,137.6} = 3.59$ ,  $P = 0.024$ , Figure 5.6). When the Photo Goat was dominant, there was no effect of ArousalIm on AsymLeft ( $F_{1,137.6} = 3.59$ ,  $P = 0.615$ ; Figure 5.6).

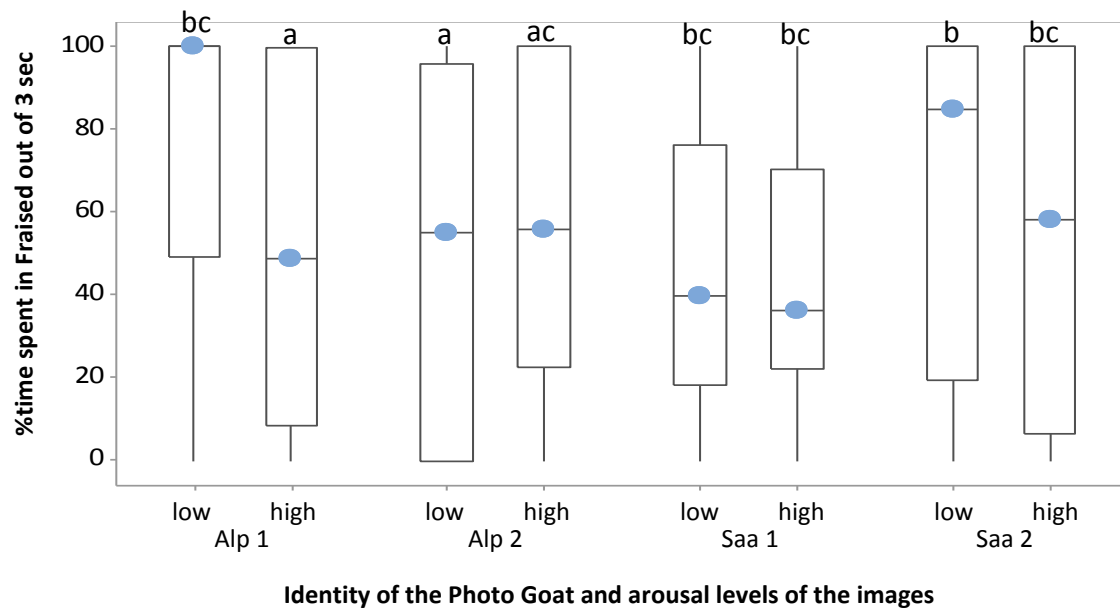


Figure 5.5. Effect of the interaction between the determined arousal of the situation in which the image of a face was taken (ArousalIm) and the Identity of the Photo Goat on time spent with the ears in forward raised (F-raised) ear posture.  $P < 0.05$  when bars share no common letters. For each group, the median is indicated by the blue dot, the box represents the first and third quartiles, the upper whisker extends to the highest data value within the upper limit and the lower whisker extends to the lowest value within the lower limit.

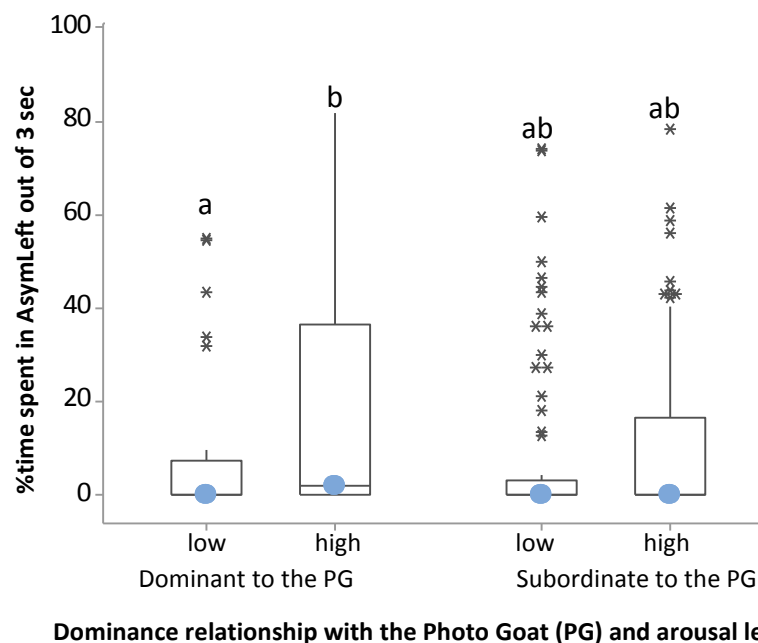


Figure 5.6. Effect of the interaction between the determined arousal of the situation in which the image of a face was taken (ArousalIm) and the dominance relationship between the test goat and the Photo Goat (RelPG) on time spent with the ears in AsymLeft postures. 'Dominant': the test goat was dominant to the Photo Goat, 'Subordinate': the test goat was subordinate to



the Photo Goat.  $P < 0.05$  when bars share no common letters. For each group, the median is indicated by the blue dot, the box represents the first and third quartiles, the upper whisker extends to the highest data value within the upper limit and the lower whisker extends to the lowest value within the lower limit. Values noted by an asterisk beyond the whiskers are outliers.

ValenceIm had an effect on the time spent with the ears backward ( $F_{2,73.8} = 4.9$ ,  $P = 0.010$ ). When the image was from a situation of negative valence, goats spent less time with the ears backward compared to a situation of neutral valence (ValenceIm = -1,  $4.7 \pm 13.9\%$ ; ValenceIm = 0,  $7.6 \pm 16.0\%$ ;  $F_{2,73.8} = 4.9$ ,  $P = 0.003$ ). There was no significant difference in time spent with the ears backward between negative and positive images ( $F_{2,73.8} = 4.9$ ,  $P = 0.103$ ). The interaction between ValenceIm and RelPG had an effect on the F-pointed ear posture ( $F_{2,108.7} = 3.92$ ,  $P = 0.023$ ). When the Photo Goat was dominant, ValenceIm had no effect on F-pointed, but when the Photo Goat was subordinate, goats spent longer in F-pointed when the images were from a negative situation compared to images from neutral ( $F_{2,108.7} = 3.92$ ,  $P = 0.017$ ) or positive situations ( $F_{2,108.7} = 3.92$ ,  $P = 0.015$ ) (Figure 5.7).

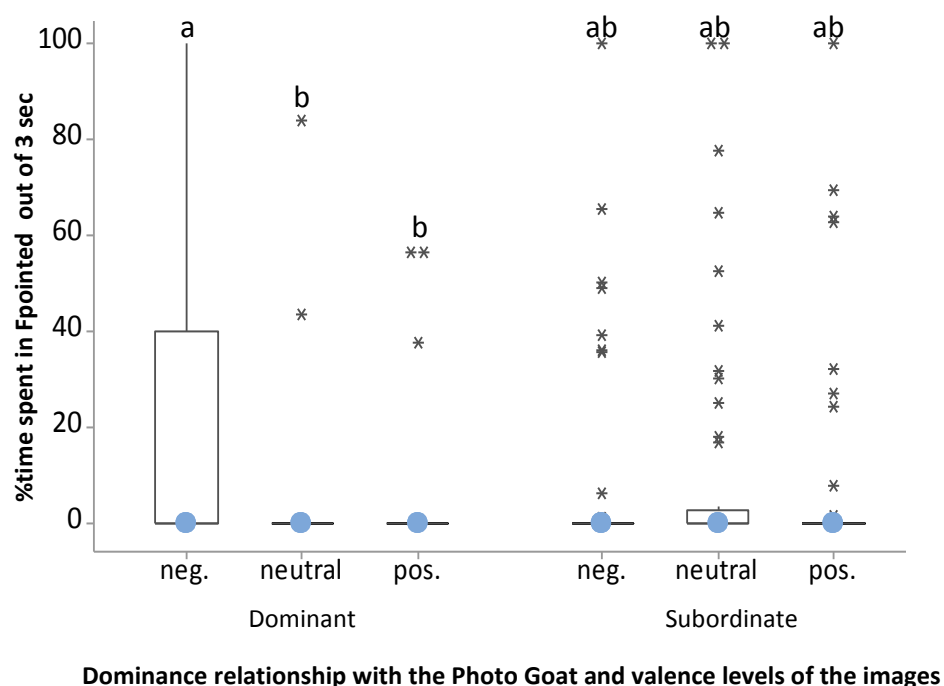


Figure 5.7. Effect of the interaction between the dominance relationship between the test goat and the Photo Goat (RelPG) and the determined valence of the situation in which the image of a face was taken (ValenceIm) on time spent with the ears forward pointed (F-pointed). 'Dominant': the test goat was dominant to the Photo Goat, 'Subordinate': the test goat was subordinate to the Photo Goat. For each group, the median is indicated by the blue dot, the box represents the first and third quartiles, the upper whisker extends to the highest data value within the upper limit and the lower whisker extends to the lowest value within the lower limit. Values noted by an asterisk beyond the whiskers are outliers.

The type of previous image affected the goats' spontaneous reactions through its valence component. Goats displayed less AsymLeft ear postures when ValencePrevIm was positive compared to when ValencePrevIm was neutral or negative or when there was no previous image ( $F_{2,143.3} = 4.09$ ,  $P = 0.019$ , Figure 5.8a). ValencePrevIm also tended to have an effect of the total number of ear postures changes ( $F_{3,149} = 2.48$ ,  $P = 0.063$ ).

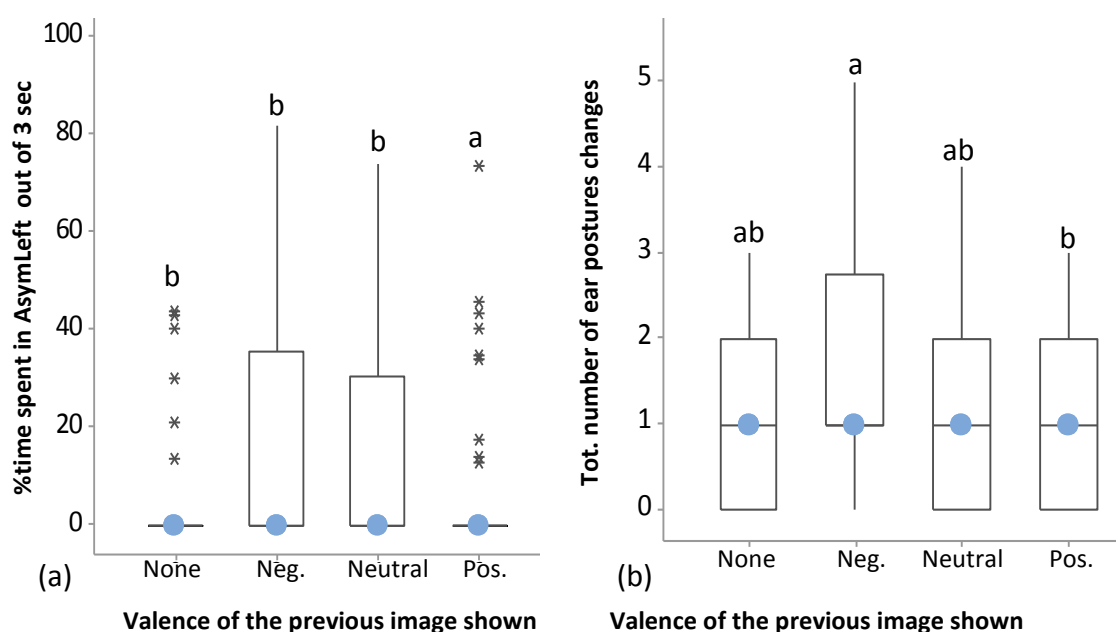


Figure 5.8. Effect of the determined valence of the situation in which the previous image presented had been taken (ValencePrevIm) on time spent with the ears in AsymLeft ear posture (a) and on the total number of ear postures changes (b).  $P < 0.05$  when the bars share no common letters. For each group, the median is indicated by the blue dot, the box represents the first and third quartiles, the upper whisker extends to the highest data value within the upper limit and the lower whisker extends to the lowest value within the lower limit. Values noted by an asterisk beyond the whiskers are outliers.

The identity of the Photo Goat also had a direct effect on backward ear postures. Goats observing images from Alpine 1 and Saanen 2 spent a shorter time with their ears backward compared to those observing Alpine 2 and Saanen 1 ( $F_{2,42.2} = 4.54$ ,  $P = 0.016$ ; Alpine 1:  $3.9 \pm 12.5\%$ , Alpine 2:  $9.3 \pm 16.0\%$ , Saanen 1:  $13.6 \pm 24.4\%$ , Saanen 2:  $4.0 \pm 12.5\%$ ). RelPG also directly affected RHM, with goats displaying more RHM when the Photo Goat was dominant to them compared to when it was subordinate ( $F_{1,179} = 4.14$ ,  $P = 0.043$ ,  $2.9 \pm 0.9$  RHM vs  $2.4 \pm 1.1$  RHM for dominant and subordinate respectively). Finally, there was no effect of breed on any of the variables.

### 5.3.2 Impact of individual characteristics on spontaneous reactions

HumApproach and the success index tended to affect the time spent looking at the screen (Table 5.5). Goats with a high success index tended to spend longer looking at the screen ( $F_{1,163.8} = 3.19$ ,  $P = 0.076$ ) while goats with a lower HumApproach score tended to look at the screen for a shorter period of time ( $F_{1,164} = 3.50$ ,  $P = 0.063$ ). HumApproach and success index had no effect on any other outcome variable (Table 5.5).

Goats that were more difficult to handle in the first half of the raceway (i.e. goats with averaged HSc scores of 4 and 5) spent more time in F-raised ear postures ( $F_{4,172.1} = 4.32$ ,  $P = 0.002$ , Figure 5.9a) and displayed fewer changes in ear postures ( $F_{4,164.7} = 5.16$ ,  $P < 0.001$ , Figure 5.9b). Goats that had higher HSc scores tended to approach the screen less once the image disappeared ( $F_{4,145.9} = 2.29$ ,  $P = 0.063$ ).

Finally, the longer a goat took to reach the 'paying attention' criterion (LatCrit), the less time it spent with its ears in the AsymLeft posture ( $F_{1,127.3} = 3.91$ ,  $P = 0.050$ ). LatCrit was not included in the final model for any of the other outcome variables.

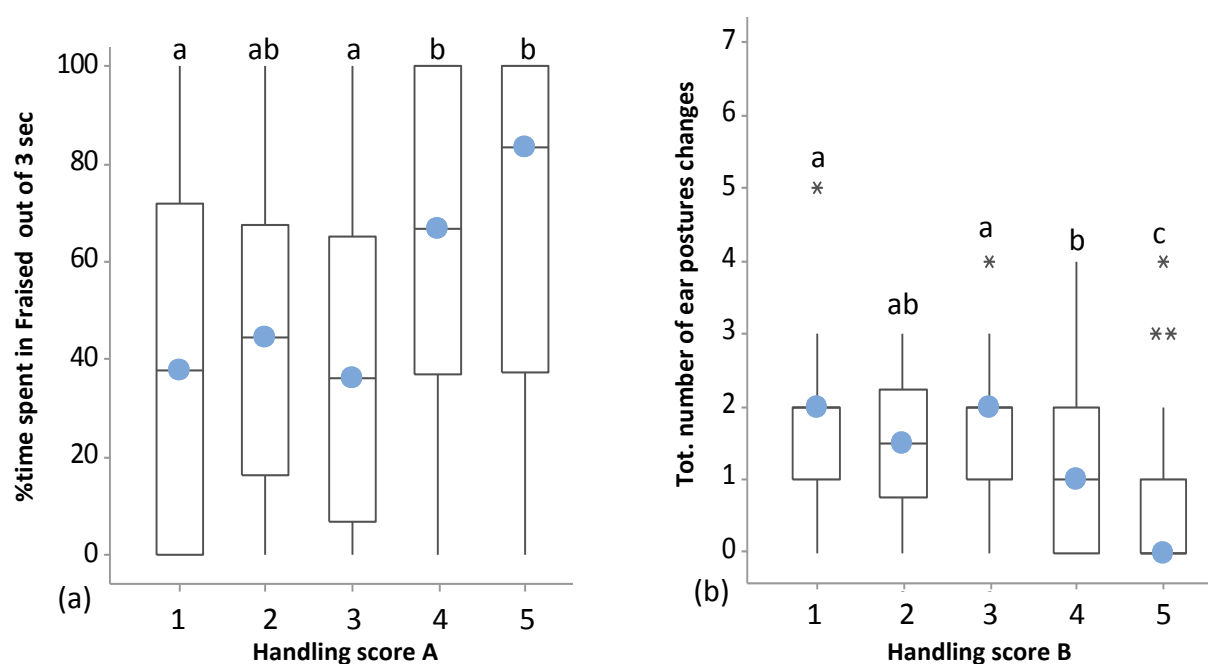


Figure 5.9. Effect of handling score (HSA, separation from the group) on time spent in forward raised (Fraised) ear postures (a) and on the total number of ear postures changes (b). HSA was scored on a 5-point scale where 1 characterised the most docile animals and 5 the less docile animals.  $P < 0.05$  when bars share no common letters. For each group, the median is indicated by the blue dot, the box represents the first and third quartiles, the upper whisker extends to the highest data value within the upper limit and the lower whisker extends to the lowest value within the lower limit. Values noted by an asterisk beyond the whiskers are outliers.

Table 5.5. Effects<sup>1</sup> of goats individual characteristics HumApproach, success index (dominance activity) and LatCrit (latency before the goat reached the 'paying attention' criterion in the observation pen) on the outcome variables and estimated regression coefficient (b)<sup>3</sup>.

Outcome variable <sup>2</sup>	HumApproach					Success Index				
	b	SEM	df	F	P	b	SEM	df	F	P
<b>F-raised</b>	-0.33	0.39	25.0	0.73	0.40	--	--	--	--	--
<b>F-pointed</b>	-0.28	0.26	66.0	1.22	0.27	--	--	--	--	--
<b>AsymRight</b>	0.27	0.16	166.0	2.62	0.11	-0.03	0.02	166.0	2.35	0.13
<b>AsymLeft</b>	-0.25	0.13	24.0	1.20	0.28	0.02	0.02	23.2	0.70	0.41
<b>Looking</b>	-0.10	0.11	164.0	3.50	0.06	0.03	0.01	163.8	3.19	0.08
<b>Changes EP</b>	0.08	0.07	25.0	1.09	0.31	--	--	--	--	--
<b>Approached</b>	0.14	0.16	118.6	0.85	0.64	-0.04	0.03	24.3	1.79	0.81

<sup>1</sup>When the parameter was not included in the final fitted model, no results are presented. None of the individual characteristics parameters were included in the final fitted models for Backward and RHM.

<sup>2</sup>F-raised: time spent with the ears in forward raised posture out of 3 s, F-pointed: time spent with the ears forward pointed, Backward: time spent with the ears backward, AsymRight: time spent with the right ear forward and the left ear backward, AsymLeft time spent with the left

ear forward and the right ear backward, Looking: time spent looking at the screen, Changes EP: total number of ear postures changes recorded, RHM: total number of rapid head movements, Approached: goat approach of the screen when leaving the video presentation pen (binomial).

<sup>3</sup>Tendencies are highlighted in italic.

### 5.3.3 Repeated exposure to the stimuli

There was no effect of session on time spent interacting with the screen ( $F_{11,180} = 0.98$ ,  $P = 0.434$ , Figure 5.10a). Within groups, neither was there any effect of session on LatCrit (Group 1:  $F_{5,90} = 0.38$ ,  $P = 0.861$ ; Group 2:  $F_{5,90} = 1.63$ ,  $P = 0.160$ ). However, there was an effect of session on LatCrit when comparing all sessions ( $F_{11,180} = 2.92$ ,  $P = 0.001$ ). Pairwise comparisons revealed that this effect was due to an overall difference between the two groups for LatCrit (Group 1:  $14.7 \pm 17.3$  s; Group 2:  $30.3 \pm 43.2$  s) (Figure 5.10b).

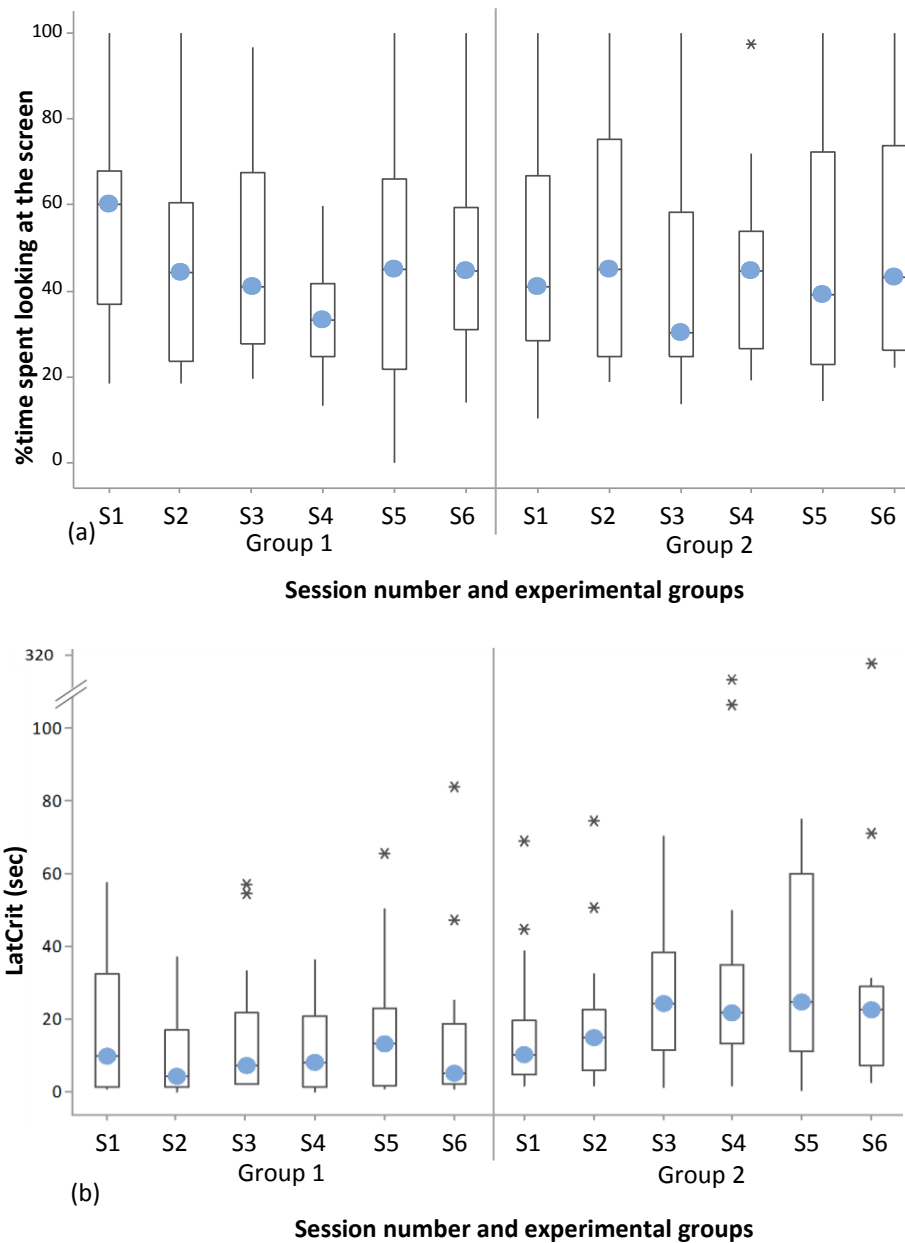


Figure 5.10. Effect of Session on time spent looking at the screen (a) and the latency to reach the 'paying attention' criterion (LatCrit) (b). Sessions from Group 1 were coded from 1 to 6 and sessions from Group 2 from 7 to 12. For each group, the median is indicated by the blue dot, the box represents the first and third quartiles, the upper whisker extends to the highest data value within the upper limit and the lower whisker extends to the lowest value within the lower limit. Values noted by an asterisk beyond the whiskers are outliers.

## 5.4 Discussion

### Spontaneous reactions to different types of images

It was hypothesised that goats would react differently to images of faces taken in situations of varying valence and arousal. Based on the results described in the previous chapter, we expected goats to distinguish images from situations of negative and positive valence, and to show higher attentiveness towards negative images.

The type of image, i.e. the unique combination of valence and arousal determined for each situation, did not have a direct effect on the goats' behaviour. However, the valence and arousal characteristics separately affected the goat's spontaneous reactions to the images. Specifically, we found that goats spent less time with the ears backwards when exposed to negative images (Ice, Resignation) compared to neutral (Rumination, Novel Object) or positive images (Food, Grooming). This was surprising, since backward ear postures have often been associated with negative situations in ungulate species. For instance higher proportions of ears backward have been related to pain after tail-docking and castration in lambs (Guesgen et al., 2016), to social isolation and exposure to unpredictable events in pigs (Reimert et al., 2013) and to isolation stress and feed-frustration in goats (Briefer et al., 2015). However, in sheep, lower percentages of ears backwards have been observed when animals were exposed to a physical stimuli of negative valence (pricking) than to a positive stimuli (kneading) (Vögeli et al., 2014b). Similarly, a higher proportions of backward ear postures was observed during pleasurable grooming in dairy cows (Proctor and Carder, 2014). The meaning of backward ear postures thus seems to be context-dependent, and more study is needed to determine if backward ear postures can be more definitely associated with the valence of a situation. Ears postures were observed

and analysed with a sum-one constraint and were thus not independent. It is possible that these changes in ear backwards were associated with changes in other ear postures, but there were no significant differences between types of images or between arousal and valence characteristics in the other ear postures recorded to confirm this hypothesis.

Goats displayed more RHM when exposed to images taken in situations of high arousal (Food, Ice, and Novel Object). A positive association between frequency of RHM and arousal has been shown in goats (Briefer et al., 2015). This would suggest that looking at images of faces taken in situations of high arousal elicited an increase in arousal in the test goats, compared to looking at images of faces in situation of low arousal.

The increase in RHM when the Photo Goat was dominant also indicates an increase in arousal for the test goat and could agree with our hypothesis that goats would be more reactive towards images of faces of dominant animals (Hewitson et al., 2007). This increase in RHM does not, however, suggest that goats were more attentive towards faces of dominant Photo Goats. More importantly, goats' spontaneous reactions to images of faces were mostly affected by interactions between characteristics of the images and characteristics of the Photo Goat, either its identity or its relative dominance status. Time spent with the ears in F-pointed and AsymLeft postures, that are associated with increased attention and vigilance (Chapter 4, Section 4.4.1, Reefmann et al., 2009a; Briefer et al., 2015), varied according to the valence and arousal levels of the images but only when the Photo Goat was subordinate, which contradicts our original hypothesis and might seem surprising at first. Expression of emotions varies depending on the social context. In sheep for instance, reactions to novel stimuli are stronger if the companion sheep is subordinate and weaker than if



the companion sheep is a dominant animal (Greiveldinger, 2007). Based on the analogy drawn between dominance/submission in animals and pride and shame in humans (Weisfeld and Goetz, 2013), this has been interpreted by Greiveldinger et al. (2007) as the dominant animal expressing its emotional reactions to stimuli more freely than subordinate sheep, as if it was not interested in the subordinate “judgement” of its reactions. From that perspective, it is likely that goats presented with images of a subordinate Photo Goat were more reactive than when faced with images from a dominant individual. This assumption implies that test goats identified the Photo Goat, which seems reasonable considering the effect of the identity of the Photo Goat. Finally, it should also be pointed out again that the repartition between subordinate and dominant goats to the Photo Goats was quite unbalanced (24 subordinate, 8 dominant). If this still allowed us to draw interesting conclusions, the generalisation of our results to the entire population of goats should only be carefully made, and further studies with a more balanced (as far as the flock hierarchy will allow!) group are necessary.

It is interesting to note that arousal level only impacted F-raised postures when images of Alpine 1 were shown. According to the preliminary dominance tests, Alpine 1 was not dominant to more test goats than other Photo Goats. Alpine 1 was nonetheless the Photo Goat with the highest success index, indicating a high dominance activity within the herd. However Saanen 1 had a very similar success index, and was thus assumed to have had a dominance activity equivalent to Alpine 1. Since there was no difference in reactions to images with varying levels of arousal in test goats exposed to Saanen 1, the higher success index could thus not entirely explain the difference observed for Alpine 1. Since a given test goat always looked at images from the same Photo Goat, the effect of Photo Goat was compared between individuals rather than a within animal. Additionally, a potential breed effect cannot be completely ruled out, since each test goat only looked at images of Photo Goats from its own breed. Now that

goats' reactions to images of faces are better known, it would be interesting to modify this part of the experimental design. The impact of the identity of the Photo Goat could be assessed by presenting test goats with images of not one, but different Photo Goats, taken in the same situation.

Our results show the importance of the interplay between emotions and social relationships. Emotions cannot be separated from their social context, and this should be taken into account when studying animals' emotions.

### Impact of individual characteristics

The two variables (HumApproach and success index) that were calculated on the basis of the characterisation tests only weakly affected goats' reactions to images of faces. It is nonetheless surprising that their success index did not affect the goats' reactions, especially considering the importance of dominance relationships in goats (Barroso et al., 2000). However, it is possible that the impact of the dominance activity of an individual in the herd, as quantified by the success index, was overcome by its dominance relationship with the Photo Goat, as a very dominant animal in the group (i.e. winner of many displacements at the feed trough) could still be subordinate to a specific individual. It is this pairwise relationship that would then influence its reaction to expressions of emotions.

Both HAT and the observation at the feed face were performed at least three weeks before goats were presented with images of faces. It is possible that the characterisation tests took place too far in advance to be relevant at the time of tests with images of faces. The human-animal relationship (HumApproach) especially could have changed due to the increased frequency and duration of human handling during the habituation phase. Furthermore, the effect of handling scores on the goats'

reactions highlights the influence of short-term human-animal interactions rather than the assessment of human-animal relationship weeks prior to the test. We found that handling scores affected the intensity of responses but not their direction. When separation from the group had been fairly difficult (high HSa scores), for instance, goats displayed behaviours indicating increased arousal and higher attention (higher proportion of F-raised ear postures and more changes in ear postures). Goats with high HSb scores, that presumably found entering the narrow video presentation pen difficult, were more likely to approach the screen. This increased interest could be linked to the reassuring effect of the presentation of images of familiar conspecifics that has been observed in situations of social isolation (Bouissou et al., 1996; da Costa et al., 2004).

In humans, personality traits such as extraversion and neuroticism have been shown to impact face-based perception of emotions (Calder et al., 2011). Including a more detailed assessment of personality by carrying tests evaluating different personality traits and repeated at two or more points prior to the presentation of images could be the next step in the assessment of perception of emotions in goats. Personality dimensions such as exploration, neophobia, anxiety or emotional reactivity could be combined with dominance evaluation to offer a more complete picture of each individual goat. Since, by definition, personality components are stable over time (MacKay and Haskell, 2015), it is likely that personality would have more impact on goats' perception of emotional stimuli than less stable parameters such as human-animal relationship or dominance activity. It would also be another important factor to control for in experimental designs.

## Methodological points

The analysis of ear postures is based on definitions of set ear postures. How precisely these ear postures are defined and reliably distinguished by human observers can appear prone to subjectivity, unless automated tracking techniques are used (Vögeli et al., 2014a). Here we aimed to refine the ethogram used in previous work by incorporating additional discrete ear postures. Our results demonstrate the importance of using a more detailed ethogram, as the two forward ear postures that we distinguished proved to be influenced by two different aspects of the emotional state displayed on the images. F-raised was affected by the arousal levels while F-pointed was affected by the valence of the situation in which the image had been obtained. Similarly, discriminating between two types of asymmetrical ear postures was critical in showing that AsymLeft was affected by arousal, while AsymRight was not. Asymmetrical ear postures can be indicative of brain asymmetries in emotion perception (Basile et al., 2009). The right brain hemisphere bias for the processing of novel and threatening stimuli is well established in mammals (Rogers, 2002; Austin and Rogers, 2014). Faces of conspecifics in a situation of high arousal could be considered an important stimulus from a risk-monitoring point of view. The left ear bias observed would thus be linked to a right hemisphere specialisation for the evaluation of potential threats in the environment (Found, 2016). Finally, it should also be taken into account that in our study the percentage of time spent in backward ear postures was fairly low (20% on average), which represents an actual duration of less than 1 second. . The percentages of time spent with asymmetrical postures were even lower ( $\leq 10\%$  on average), which is in agreement with observations made by Briefer et al. (2015). As such our results need to be treated with caution.

Valence and arousal of the previous image shown were included in the analysis, to identify potential carry-over effects. However, it was only the valence level of the previous image that affected the reaction of the goat. Test goats displayed more RHM, which are indicative of higher arousal, after previously seeing an image from a situation of negative valence. Conversely, following presentation of an image taken in a situation of positive valence, test goats displayed less AsymLeft ear postures, which could be linked to a lower vigilance. This might indicate that goats formed expectations based on the previous image shown: after seeing a negative image, goats were more vigilant and conversely they were less vigilant after seeing a positive image. More research is needed to confirm this hypothesis.

Another aim of this study was to improve the design of the experimental set-up, in response to the results and our observations from the previous study with goats (Chapter 4). In the previous study, the response to the first session of presentation of images was notably different from the others in terms of behaviours reflecting attention towards the screen. We attributed these differences to the effect of novelty, since the first session was the first time the goats had seen the screen lit up. To prevent this influence of novelty, a sham test session was included in the habituation phase of the present study. This proved to be successful, since there was no longer any difference between Session 1 and the following sessions within each group for either LatCrit or time spent looking at the screen. However LatCrit was overall longer for Group 2 than for Group 1. The two groups had been balanced as far as possible for breed, human-animal relationship and dominance relationship between test goat and Photo Goat but other environmental factors, such as weather (sunny and dry for Group 1, rainy and windy for Group 2), or uncontrolled noises outside the test arena could also explain this difference.

## 5.5 Conclusion

Valence and arousal levels induced in the situations used to produce images of faces independently affected reactions of goats to the images. Most importantly, spontaneous reactions of goats to images of faces were affected by the interaction between the dominance relationship or the identity of the Photo Goat and the valence or arousal levels. Goats were more reactive when viewing images from subordinate Photo Goats. This study proves the importance of the interplay between social relationships and emotions, and demonstrates the need to take social context into account when investigating the expression and perception of emotions.

## Chapter 6: General discussion

## 6.1 Introduction

The assessment of emotional states in animals has seen considerable improvement in the past decade and notably through the development of new methods such as cognitive bias testing that enables researchers to infer the emotional state of the animal. However, the study of animal emotions has been primarily focused on the individual, and social aspects of emotions have rarely been addressed, especially in farm species (Spinka, 2012). Through their expressions, emotions are social signals, and as such can be perceived by conspecifics. To implement a truly global approach of animal welfare, it is essential to improve current knowledge about how emotions are perceived by others, and which factors, such as the emotional state of the observer, its dominance status or individual characteristics, affect this perception.

This PhD project aimed to further understand how small ruminants perceive the emotions of conspecifics. Face-based emotion perception was addressed more specifically, as the second aim of this project was to determine whether images of faces could be used as cues in judgement bias tests. If that were the case, it would indeed offer a solution to some of the limitations of judgement bias methods, mostly related to the extensive training phase necessary. The studies described in Chapters 2, 4 and 5, were specifically directed at the perception of facial cues in both sheep and goats. In Chapter 3, the effect of the emotional state of the individual on its perception of emotional stimuli from conspecifics was addressed, using videos of conspecifics in situations of varying valence.

## 6.2 Discriminating between facial expressions of emotions

The first step in the evaluation of the potential use of images of faces for judgement bias studies was to assess whether sheep and goats could discriminate between images of faces displaying different emotional states. To that end, the faces of



sheep and goats (Photo Sheep and Photo Goats) were filmed in situations inducing emotional states of varying valence and then presented to a sample of familiar test animals. The ability to discriminate was assessed both in a simultaneous discrimination task (Chapter 2) and by testing whether there were differences in spontaneous reactions to images of faces (Chapter 4). In Chapter 2, sheep learned to discriminate between images of faces taken in a neutral (ruminating) and in a negative (social isolation or aggressive interaction) situation, and to transfer this rule to images of faces of different individuals (for social isolation and rumination images, not aggressive interactions). In Chapter 4, goats showed differences in their spontaneous reactions to images of conspecifics taken in a positive (grooming) or in a negative (ice pack applied to the udder) situation, indicating that they were distinguishing between the two types of facial expressions. From these two sets of results, it was concluded that sheep and goats could discriminate between facial expressions of emotions.

### 6.3 Perception of emotions

The main advantage of using images of faces in judgement bias tests is that faces displaying emotional states could have an intrinsic valence. If animals perceive the valence of biologically relevant cues, then extensive discrimination training would not be necessary. The second step was thus to evaluate whether small ruminants not only distinguished between differences in facial features, but also perceived the emotion displayed in the image.

In Chapter 2, differences were observed in the learning speed of sheep, depending on the type of image paired with a reward. Sheep that had to learn to associate an image of a face taken in a negative situation with a food reward learned faster than sheep that had to learn the association between a food reward and a neutral face. This is consistent with the overall attentional bias for negative stimuli that has

been observed across taxa (Carretié et al., 2001) and indicates that these images were indeed perceived as negative stimuli by the sheep. Similarly, goats were more attentive towards images of faces of conspecifics taken in negative situations (Chapters 4 and 5), which also indicates that the valence of the facial expressions was perceived. The images of faces used in Chapter 5 made it possible to separate the influence of the arousal from that of the valence of the situation in which the image was taken on the perception of the goats. It was demonstrated that goats reacted independently and differently to the valence and arousal levels displayed in images of faces, indicating the ability to discriminate finely between facial expressions.

Chapter 3 differed from the other experimental chapters of this thesis, since it did not involve facial stimuli, but videos where the whole animal were shown. The main aim of this chapter was to evaluate the impact of mood on emotion perception in sheep. A low-mood state was thus induced by four weeks of unpredictable housing conditions (adapted from Destrez et al., 2013b) in half of the animals. Results from this study indicated that sheep were able to discriminate between emotional stimuli in conspecifics and confirmed results from the study presented in Chapter 2. This study also found that sheep were more attentive towards negative stimuli (videos of conspecifics in social isolation or engaged in agonistic interactions). Moreover, sheep from the negative housing conditions reacted more strongly and more negatively to negative videos, especially of aggressive interactions. This suggests that negative housing conditions induced a low-mood state in sheep that influenced their responses to emotional stimuli. The influence of mood on the perception of emotional stimuli from conspecifics means that emotion perception is subject to cognitive bias in sheep.

These first results all drew an encouraging picture towards the use of images of faces as cues in judgement bias studies. The next step was thus to evaluate responses to ambiguous stimuli, i.e. morphed images of faces. To be able to use this type of cue in judgement bias studies, animals had to i) react differently to positive and negative

images, ii) show responses to the morphed images that were intermediate to the responses to positive and negative images, iii) show gradual responses to the morphed images, e.g. with a linear positive relationship between the degree of negative emotion in the morphed photo and negative responses. The results of this study did not support these hypotheses. Responses to the morphed faces were not always intermediate to responses to negative and positive images. Moreover, there was no linear relationship between the intensity of the response and the degree of negative emotional state in the image presented. In that light, the use of morphed images as ambiguous cues in judgement bias in goats may be inappropriate. The judgement bias design might not be the most adapted to the use of morphed images of faces. Faces are extremely complex stimuli, and morphing might make them difficult to interpret for animals. More research is needed to understand better how images of morphed faces are perceived.

Nonetheless, it would be a mistake to dismiss the use of faces as cues in cognitive bias studies. Biologically relevant stimuli have been successfully used in cognitive bias studies involving attention bias. These studies take advantage of innate biases in attention towards certain stimuli such as faces in rhesus-macaques (Bethell et al., 2012) or threatening stimuli in sheep (Lee et al., 2016). Attention bias studies might be a better indicated methodological framework to make the best use of biologically meaningful stimuli such as faces.

## 6.4 Social dimension of emotion perception

Across Chapters 3 and 4, the influence of the identity of the Video Sheep or Photo Goats was clear. In both studies, reactions of the test animals varied depending on 'who' was on the screen. The main hypothesis proposed to explain the variation in reactions was an influence of the dominance relationship between test animals and Video Sheep/Photo Goat. Sheep have previously been shown to be more attentive

towards dominant conspecifics (Hewitson et al., 2007). Unfortunately in the studies presented in Chapters 3 and 4, information about the dominance relationship between test animals and stimuli animals had not been collected and thus no conclusions could be reached. Therefore, in the final study of this project (Chapter 5), the relationship between the Photo Goats and the test goats exposed to images of its face were established prior to the presentation of images. There was no difference in attention (time spent looking at the screen) that related to whether the Photo Goat was dominant or subordinate to the test goat. The test goats, however, reacted more strongly when faced with images from subordinate Photo Goats. This might appear surprising at first, but in ruminants, sheep have been shown to display stronger reactions to novel stimuli when paired with a subordinate sheep than when paired with a dominant sheep (Greiveldinger et al., 2007). Dominance explained some of the variability observed in the goats' reactions to images of faces, but not all of it, and the identity of the Photo Goat still affected certain behaviours (time spent with the ears backward and forward raised) without a clear link with the dominance status of the Photo Goat. This variation could be caused by other types of relationships such as affinity between the two individuals or sociability of both the Photo Goat and the test goat (Sibbald et al., 2005).

Results described in Chapters 3, 4 and 5 demonstrate the importance of the interplay between emotions and social relationships. The study of emotions, and especially of the perception of emotions, cannot be separated from their social context. When working with images or videos of familiar conspecifics, it is essential to form a precise understanding of the relationships within the group of subject animals and to characterise individuals as much as possible prior to the presentation of any emotional stimulus.

On a wider note, the importance of the social dimension of emotions cannot be over-estimated. This describes any perception by others of the emotional state of an

individual, and the fact that this perception can affect their emotional state (Spinka, 2012). From an animal welfare point of view, taking into account the social dimension of emotions is crucial. Emotional contagion, i.e. the shifting of an animal's emotional state towards the emotional state perceived in a conspecific (Spoor and Kelly, 2004) could have consequences for animal welfare. To underline the relevance of emotional contagion for animal welfare, it can be presented simply: what if it took only one (un)happy sheep to make the entire flock (un)happy? Furthermore, if, as suggested by the study presented in Chapter 3, animals in a negative emotional state have an altered perception of social emotional stimuli, their ability to avoid aggression or painful situations, establish social bonds or integrate into a social group may be compromised.

## 6.5 Development of a new method

Studying emotions is no easy task, and studying emotions in combination with highly complex stimuli such as faces makes it an even more fascinating challenge. Images of faces are interesting for judgement bias studies because of the intrinsic value of facial expressions (valence and arousal levels). New methods were needed to investigate the use of images of faces and the development of a valid method to assess the behaviours of sheep and goats in response to images of faces was an important part of this project.

In Chapter 3 to 5, experimental situations were designed where the valence and arousal dimensions of the emotional states induced could be evaluated. To determine the emotional state elicited in animals, a cross-modal approach is often used (physiological and behavioural (Reefmann et al., 2009c; Briefer et al., 2015)). However, in well studied species such as sheep and goats, behavioural and physiological markers of emotional states have been mapped, and it is possible to use only a behavioural approach (Vögeli et al., 2014a). Moreover, our evaluation of the emotional state

induced in the Photo/Video animals was based on the functional framework of emotions proposed by Mendl et al. (2010). In that framework, stimuli enhancing fitness or rewarding stimuli are thought to elicit positive emotional states (presentation of food, grooming), while fitness-threatening or punishing stimuli (social isolation, aggression, discomfort) elicit negative emotional states. This is similar to the approach used in goats by Briefer et al. (2015), and we consider that this is a valid method for those two species (but cross-modal validation might be needed in less studied species). It can be noted however, that providing more quantified measures of behaviours during the filming situations would have consolidated our findings, as would have the use of physiological measures such as heart rate (for instance, in sheep and goats, elevated heart rate has been reported during isolation stress (Reefman et al., 2009; Briefer et al., 2015)).

If animals have never been exposed to images of conspecifics, they are more likely to confuse the physical image and the subject shown in that image and consider the image as a real animal (Parron et al., 2008). For instance, sheep have been shown to display social behaviours towards images of conspecifics such as sniffing and licking the image (Bovet and Vauclair, 2000). The model of processing of repeatedly experienced pictorial stimuli presented by Fagot et al. (2000) proposes three levels in the processing of images: Confusion, where the animals consider the image of the item as the real item, and which is considered the starting mode of this dynamic model. The two possible levels following from that starting mode are Independence, where the animals process the image as a set of features with no relations to the item photographed, and Equivalence, where the image is processed as a representation of the item. There is thus a risk that by repeatedly exposing the animals to images of faces, as during training for a discrimination task, the stimuli will lose their intrinsic meaning and be perceived as merely sets of features instead of facial expressions of emotions (Fagot et al., 2010). Preserving the naivety of animals exposed to images is thus

essential and is typically achieved by not exposing animals to too many images or to the images for a long time. This is why the animals were exposed to very brief presentation of emotional stimuli in the studies described in Chapters 4 and 5. The 3 seconds presentation duration, used in these experiments, can be considered to be a very short interval over which to conduct behavioural observations. However emotions are by definition of short duration (Désiré et al., 2002), and facial displays of emotions are brief and typically last between 0.5 and 2 sec in humans (Shreve et al., 2009). Moreover facial expressions are graded and dynamic signals (Waller and Micheletta, 2013) so presenting facial expressions for a very short time is thus closer to mimicking real life situations. In human neuroimaging studies of face-based emotion recognition, stimuli are presented for similarly short periods of time,  $\leq 3$  sec (Lee et al., 2008a). The results described in Chapters 4 and 5 showed that such short presentation times and observation periods were sufficient to induce different behavioural reactions and to observe those differences. Highly changeable behaviours (up to four changes in ear postures were observed during the 3s periods) such as ear postures (Vögeli et al., 2014a) were especially suited for such small observation intervals.

In Chapter 4, the first session of presentation of images was notably different from the others for behaviours indicative of the attention of goats towards the screen. These differences were attributed to the effect of novelty of the stimuli, since the first session was the first time the goats had seen the screen lit up. To prevent this influence of novelty, a sham test session was included in the habituation phase of the study presented in Chapter 5. During this sham session, only a grey inverted triangle was presented on the screen, mimicking the shape of a goat head. Adding this session to the habituation phase proved successful, since no difference were observed between the first session and the following sessions within each group for behaviours indicative of attention towards the screen (Chapter 5). Other modifications were made to the set-up,

such as the addition of a small 'video observation pen' that limited goats' exploration of the test pens, so that their attention could be more quickly directed towards the screen.

The experimental set-up presented in Chapter 5 is thus the outcome of three years of reflection based on many observations, trials and errors. It is a suitable apparatus for studying spontaneous reactions of goats to images of faces of conspecifics, and its use could be extended to sheep.

## 6.6 Limitations and perspectives

### 6.6.1 Sample sizes

Unfortunately, throughout this project, no sample size calculations were used to determine how many animals should be included in our experiments. This is mostly because the number of animals that could be recruited depended on very practical limitations, such as the number of animals of a given age present on the farm. However, it would have been a useful addition to the work presented here, and would have provided a solid consolidation of our findings, especially about their potential generalisation to the whole population of sheep or goats. This is something that will be taken into account in further work, as the use and spread of such practices in scientific research is essential to its quality.

The numbers of Photo/Video Animals could used could also be questioned. Since we were limited by the total number of tested animals, we had to limit our number of Photo/Video Animals, so that each Photo/Video Animal would be observed by enough test animals. For instance, in Chapter 4 and 5, if we had used eight Photo Goats instead of 4, each Photo Goat would have been observed by only four test goats, and we would not have been able to conclude whether differences in reactions were caused by the different facial expressions, or by the identity of the Photo Goat. Another



possibility would have been to use different images of the face of the same Photo animal for every test animal. However, with a different photo for each test animal and for each type of image, if there had been no significant differences between the animals' reactions, we would not have been able to identify if this was due to the different photos, the different emotions, or the different Photo Animals. Because we were at such an early stage of investigating face-based emotion recognition in sheep and goats, we chose to limit variability as much as possible to avoid inconclusive results. We do realise that this makes our results less generalizable. Since differences in reactions to the different types of images/videos were identified, it might make the experimental designs look too conservative retrospectively, but this was a precaution we thought we had to take. In future studies, we would most likely consider using several photos from each Photo Animal for the same type of image.

In this thesis we have been reporting tendencies as well as significant results. This is something that is hotly debated in science at the moment, and reasonable arguments can be found on both sides. We consider that when tendencies are biologically meaningful, and reasonably strong, it is interesting to report them, for the general interest of the reader, and to indicate effects that should be taken into account in the experimental designs of further studies. Considering the potential sample size issue discussed above, it could be considered that such tendencies should not be reported, and some previously presented results have indeed been reconsidered in this corrected version. However we still regard the tendencies presented in this thesis as biologically meaningful and of interest, provided that their interpretation remains careful and do not draw generalised conclusions too easily.

## 6.6.2 Standardisation of stimuli

When filming or photographing animal faces, the quality of the images obtained can vary, for instance lighting can influence tones, and sharpness or brightness can

differ. Orientation of the head and the camera angle can also change between images due to the movements of the animal. To this variability is added the natural complexity of facial expressions. The intensity but also style of facial expressions can vary between and within individuals (Parr, 2003; Waller and Micheletta, 2013). Obtaining standardised images of faces of sheep and goats was definitely one of the main challenges faced during this project. If the results presented in this thesis seem to indicate that it was met fairly successfully, there is still room for improvement and a few possible directions for future research are presented below.

First, to account for variability in facial expressions, the development of tools such as Facial Action Coding Systems (FACS) could be extremely useful. FACS split up facial expressions into actions units, each corresponding to the contraction of a specific facial muscle. With FACS, variation in intensity and shape of facial expressions can be quantified. After being developed in humans (Ekman and Friesen, 1978) and in primate species (Vick et al., 2007; Parr et al., 2010), FACS has been extended to domestic species such as horses (Wathan et al., 2015). Being able to quantify the variability in facial expressions would allow researchers to describe precisely the repertoire of facial expressions in sheep and goats. Developing SheepFACS and GoatFACS would give researchers the ability to precisely choose the images of faces used and to work with standardised facial expressions across individuals. The next step would then be to identify precisely which expressions are associated with specific emotional states, and in which situations. This would need to be done across breeds to ensure the validity of the generalisation. Specificities such as the presence of horns should also be taken into account. The number of animals included in this project would also need to be established beforehand. In a first phase, standardised situations would be designed, based on the literature, and validated with cross-modal measurements (behavioural and physiological) to determine the emotional state induced in the animal. Then these situations would be replicated and HD videos of the faces of the animals would be

taken, so that they could then be analysed by trained observers, using the FACS systems previously developed.

Even if a SheepFACS would give access to a finer comprehension of facial expressions, defining the experimental situations in which these expressions can be recorded can be challenging. Firstly, recording facial expressions that are associated with social interactions without interfering with the animals can be difficult. In Chapters 2 and 3, an artificial competition situation was used to induce agonistic interactions, but this cannot be easily applied to affiliative situations or to play, for instance. More generally, obtaining images of faces displaying positive emotions is not as straightforward as with negative emotions, mostly because expressions of negative emotions are much more intense than of positive emotions (Boissy et al., 2007). In Chapters 2 and 3, despite the systematic desensitisation, it was not possible to use a positive interaction with a human handler (grooming) to obtain images of faces, which is why only neutral and negative situations were used. Separating a sheep from the flock or imposing close human contact would have been too stressful at that stage for the animals involved in these studies. Using food rewards within the group as a positive situation was also not an option, since competition was high between animals and made filming individual faces very difficult. Obtaining images of faces in positive situations would most likely be possible after an extended habituation period of Scottish Mules ewes, but also maybe with a tamer lowland breed, or dairy animals.

The solution developed by Parr et al. (2003) in chimpanzees was the creation of 3D-models of faces where the different Action Units described in the FACS could be activated independently to create perfectly standardised facial displays. Whether similar methods could be used in small ruminants is yet to be determined through future research.

### 6.6.3 Physiological measurements

Physiological measures such as heart rate, heart rate variability, respiration rate and haemodynamic changes in the cortical brain have been linked to emotional reactions in sheep and goats (Reefmann et al., 2009b; Vögeli et al., 2014b; Briefer et al., 2015). Physiological parameters could have been a useful addition to the behavioural data collected, to corroborate the arousal and valence levels determined for each situation used to obtain the images or videos stimuli. Heart rate, for instance, has been strongly correlated to arousal (Briefer et al., 2015) and would have been a good indicator to quantify the arousal levels induced by the situations the images were taken in. In Chapter 3, the effect of negative housing conditions on sheep was assessed through measurement of faecal glucocorticoids concentrations, but no differences were detected between the control and treatment group. Leukocyte counts have been shown to decrease in sheep exposed to similar treatments and might have been a more sensitive indicator (Destrez et al., 2013b). Furthermore, negative housing conditions have been shown to induce differences in the physiological responses of sheep to emotional stimuli (Doyle et al., 2011; Vögeli et al., 2015). For instance sheep housed in negative conditions had higher heart rates than sheep from enriched housing conditions (Reefmann et al., 2012). After preliminary measurements of baseline heart rate for all sheep, heart rate could have been recorded before and during the 30s duration of exposure to the videos, to evaluate differences between the control and treatment groups in changes induced by the presentation of the emotional stimuli.

However, including physiological measurements is not always advantageous or even appropriate. Considering the resting heart and respiratory rates of goats (70-80 beats and 15 to 30 breaths per minute respectively (Reece, 2015)), physiological measurements such as heart or respiratory rate were not indicated to assess the reaction of goats presented with images for 3s only (Chapters 4 and 5), especially when the majority of the animals did not look at the screen for 3s. Moreover, the correlations

between physiological measures and ear postures established by Reefman et al. (2009b) indicate that physiological data are not an absolute necessity and this work provided a validation that the fine recording of ear postures can be used for assessing emotional states in sheep and goats.

#### 6.6.4 Perspectives for future research

The main aspect of the perception of emotions studied in this project concerned faces and facial expressions, but expression and perception of emotions are multimodal processes. Indeed, the expression of emotions also involves changes in body posture, in vocalisations or in odour, and these changes can also be perceived by conspecifics (Boissy et al., 1998; Siniscalchi et al., 2013; Briefer et al., 2017). A more global approach of emotional signals would integrate these different modes of expression and, experimental stimuli could, for instance, combine images of facial expressions with vocalisations. Furthermore, facial muscles do not move from one set expression to the next, but involve constant movement and facial expressions are dynamic stimuli (Waller and Micheletta, 2013). Presenting sheep and goats with short videos of faces instead of 2-D images may be more appropriate, and would be particularly indicated for expressions including vocalisations, as shown in chimpanzees by Parr et al. (2003). This type of multimodal approach would also be beneficial to our understanding of the social dimension of emotional states in animals and of the propagation of emotions within a group.

Finally, this thesis presented studies on the perception of emotions in sheep and goats, but no comparison between the species was drawn. Cross-species studies have compared the foraging strategies of the two species (Hosoi et al., 1995), and cross-fostering species have investigated the influence of mothers on the sexual preferences of offspring (Kendrick et al., 1998). Since the aim of this project was not to compare the two species, strong comparisons cannot be drawn, as the methods used

with sheep and goats did differ. Briefly, in the work presented here, it seems that both species perceived the valence of the emotional state expressed on the images/video stimuli. Both sheep and goats also displayed higher attention towards images of faces or video of conspecifics taken in negative situation. As discussed before, this agrees with the attention bias for negative stimuli that has been identified in mammals (Carretié et al., 2001; Bar-Haim et al., 2007). In both species attention was indicated by forward ear postures (Chapter 3, 4, 5), however there seem to be variations in backward ear postures (Chapter 3 and 5), and more studies are needed to clarify the relationship between backward ears and emotional states. Even though they are closely-related species that are similar in their biology and behaviour, sheep and goats have distinct characteristics (Collias, 1956). Goats and sheep differ in their social behaviour, goats are more reactive and more aggressive than sheep and goats also display more exploratory behaviours than sheep (Miranda-de la Lama and Mattiello, 2010). Social systems and hierarchy's dynamics within a group differ between sheep and goats. Leadership seems to be more defined in sheep, which follow a single animal leader and move in compact groups, while goat herds move by forming a thin line (Escós et al., 1993). In mixed-species herds, goats have been shown to be leading sheep (Hafez et al., 1969). Considering the importance of inter-individuals relationships that we identified in this project (Chapter 5), it is likely that such differences in social behaviour could influence sheep and goats reactions to display of emotions from conspecifics. Following from the work presented here, it would also be interesting from an evolutionary point of view to clearly establish difference and similitudes in face-based perception of emotions between these two species, by testing sheep and goats in similar experimental set-ups.

## 6.7 Conclusion

The main aim of this PhD was to improve our understanding of emotion perception in small ruminants, and to evaluate the potential of using images of faces in judgement bias tests. This was achieved through four studies investigating the responses of sheep and goats to images of faces and videos of conspecifics taken in situations of varying valence and arousal. We can conclude that sheep and goats discriminate between facial displays of emotions in conspecifics and that they seem to perceive the emotional state displayed. Sheep and goats were, overall, more attentive towards individuals displaying negative emotional states. In addition, a novel experimental set-up was developed to evaluate the spontaneous reactions of animals to images of conspecifics. The results presented in this thesis highlight the importance of studying emotions in animals as communication signals and to not limit the study of emotions to the individual, but to take into account relationships between individuals, and especially dominance. The work conducted during this project contributed to the improvement of current knowledge on animal emotions.

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